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**In Vitro Dissolution Rate and Fluoride Concentration
Changes of Human Enamel Treated with
Aqueous SnF₂ Stabilized with Amine Fluoride 297**

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DEDICATION

I Dedicate This Dissertation

To My Parents.

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1. HISTORICAL

Mouth rinses have been used for thousands of years as part of cultural or religious beliefs, as a cure for halitosis and to treat oral and general diseases. Hippocrates and Pliny, respectively, recommended pepper and catorium, and urine as components in mouth washes. By the 18th Century a typical rinse contained water, alcohol, solammoniac and honey to "whiten teeth, remove encrusting tartar and to prevent dental caries (McCormick 1968). After Pasteur, Koch and Lister published their work on bacteria and infection, and Miller demonstrated the relationship between oral bacteria and caries, several mouth washes were advertised as having powerful oral antibacterial activity.

The use of acidic or alkaline mouth rinses was controversial as both forms claimed the same therapeutic effect. Mouth rinses producing nascent oxygen were also frequently prescribed. In 1939 the Council on Dental Therapeutics of the American Dental Association (CDT ADA) made a strong stand against the wild medical claims made for many brands of mouth rinses. From that point, mouth rinses could only be advocated for the cleaning of teeth. However, in 1975 the CDT ADA accepted both neutral sodium fluoride (NaF) and acidulated phosphate fluoride mouth rinses as effective agents in reducing the incidence of dental decay.

2. GENERAL

Mouth rinses and fresheners are marketed as liquids, troches, lozenges, and sprays and are used at full-strength or diluted in water. The basic components are water, alcohol, flavouring and coloring agents but astringents, emulsifiers, sweeteners, antimicrobial or other therapeutic substances may be incorporated.

Astringents added to mouth rinses are generally zinc or aluminum salts while emulsifiers, used to lower surface tension and stabilize other components include fatty acid esters or block co-polymers (polyoxyethylene and polyoxypropylene). Antimicrobial substances which deodorize by inhibiting oral bacterial activity include quaternary ammonium (cetylpyridinium chloride, benzethonium chloride), or phenolic compounds (phenol, thymol). Other antimicrobials used are metal ions (zinc, tin), hexetidine, chlorhexidine, or antibiotics (vancomycin, erythromycin). From an anti-carries point of view, therapeutic mouth rinses contain fluoride salts.

Cosmetic or therapeutic mouth rinses should neither burn nor etch the soft tissues, should not interfere with the taste sense nor stain the teeth and yet have an acceptable taste. Therapeutic mouth rinses should reduce bacterial activity as well as the amount of dental plaque and should promote the remineralisation of initial carries lesions. Several fluoride

mouth rinses, meeting some of the above requirements have been clinically tested and play a significant part in preventive dentistry in many countries.

3. INTRODUCTION

The role that water fluoridation has played in reducing caries incidence has been well documented since the early 1940's (Dean 1946; Arnold et al. 1962). There after the direct application of fluorides to enamel (topical, dentifrice, mouth rinse) were investigated, but two studies using acidified sodium fluoride (NaF) mouth rinses yielded negative results (Bibby et al. 1946; Roberts, Bibby and Wellock 1948). Unfortunately, subsequent work on fluoride-containing mouth rinses produced conflicting results. An example of a positive result with a 90% caries reduction was described by Weisz (1960) using neutral NaF (0.25%) but the method was not accepted because the recommended daily dosage was considered too high.

Several fluoride salts such as potassium fluoride (Torell and Siberg 1962), ferric fluoride (Fjaestad, Norstedt and Torell 1961) and ammonium fluoride were tested as mouth rinses (DePaola et al. 1977). In such studies the rinsing frequency varied and the fluoride concentrations (low versus high potency, respectively) ranged from 45 ppm F to 3000 ppm F (Bibby et al. 1946; Heifetz, Driscoll and Creighton 1973). Because of its excellent stability in water, neutral

taste and lack of staining NaF is the most frequently used and tested fluoride mouth rinse.

The CDT ADA has listed mouth rinses containing 0.05% NaF as accepted by the CDT ADA (1984) and the more popular brand names include:

- a) ACT^R (Johnson & Johnson; Oral Care; 501 George Str.,
New Brunswick, NJ 08903, USA)
- b) Fluorigard^R (Colgate-Palmolive Co.; 909 River Rd.;
Piscataway, NJ 08854, USA)
- c) Fluorinse^R (Cooper Care Inc.; 170 S. Whisman Rd.,
Mountain View, CA 94043, USA)
- d) Janar's Rinse^R (Janar Co.; 4615 S.E. Paris Rd.,
Grand Rapids, MI 49501, USA.)
- e) Kari-Rinse^R (Lorvic Corp.; 8810 Frost Ave.,
St. Louis, MO 63134, USA.)
- f) NaFrinse^R (Orachem Pharma.; 9990 Global Rd.,
Philadelphia, PA 19115, USA.)

Several fluoride salts reduced the in vitro solubility of enamel (Volker 1939; Volker et al. 1940; Muhler and Van Huysen 1947). Stannous fluoride (SnF_2) was among the most efficient agents in this group. During the mid-50's SnF_2 gained acceptance as an active agent in dentifrices (0.4%; 970 ppm F) and in concentrated topical solutions (8% & 10%; 20,000 & 25,000 ppm F; Muhler et al. 1956; Gish, Howell and Muhler 1957; Muhler 1958; Gillings, Broadhurst and Martin 1964; Shannon and West 1979). SnF_2 reduced rat caries

incidence significantly better than NaF (Muhler and Day 1950; Muhler, Nebergall and Day 1953) and had excellent anti-plaque properties (Lilienthal 1956a & 1956b; König 1959; Tinanoff et al. 1976; Svatun and Attramadal 1978; Beiswanger et al. 1980; Tinanoff et al. 1983). Despite its excellent anti-carries properties, SnF_2 is handicapped as a mouth rinse because of its instability in water, astringent taste and staining potential.

To overcome the problem of instability, SnF_2 is marketed in tablet form and must be freshly dissolved in water before each rinse. The CDT ADA has accepted StanCare^R (Block Co.; 257 Cornelison Ave.; Jersey City, NJ 07302, USA) which yields a 0.1% (250 ppm F) concentration. Most of the other SnF_2 preparations accepted by the CDT ADA have a 0.4% SnF_2 concentration but are gelled with glycerine.

Over the years researchers tried in vein to formulate precipitate-free stabilized aqueous solutions of SnF_2 . Initially, Lim (1970) showed that NaF, glycerol and tartaric acid kept SnF_2 solutions free of precipitates. However, Shannon (1969, 1970) stabilized SnF_2 by heating it in water-free glycerine to 150°C. Subsequently, Mühlemann and Schmid (1985) stabilized aqueous solutions of SnF_2 by mixing it with amine fluoride 297 [AmF 297; N'-Octadecyltrimethyldi-amine-N'N'N'-tris-(2-ethanol)-dihydrofluoride]. In contrast to Shannon's (1969) method of stabilizing SnF_2 , Mühlemann



and Schmid's (1985) method is simpler and any concentration of SnF_2 can be stabilized by mixing the SnF_2 with an equivalent amount of AmF 297.

The literature describing the anti-carries and anti-plaque properties of AmF 297 date back to the 1950's (Mühlemann, Schmid and König 1957; Mühlemann and Schmid 1958; Hermann and Mühlemann 1958; Mühlemann and Wolgensinger 1959; Bramstedt and Bandilla 1966; Gülzow 1966; Candeli, Marci and Fornari 1967; Pohto, Antila and Toivanen 1971; Schneider and Mühlemann 1974; Breitenmoser 1975). Oral fluoride clearance was significantly longer with an AmF 297 than a NaF mouth rinse both containing 250 ppm F (Mühlemann and Rudolf 1975). However, Ringelberg et al. (1979) reported negative clinical results in a study testing AmF 297 in mouth rinse and dentifrice forms. In that study, the SnF_2 test group also had negative results which questions the entire study.

Initial clinical anti-plaque studies with the SnF_2 /AmF 297 combination were undertaken to test the most effective molar fluoride ratio (MFR). SnF_2 and AmF 297 were tested at MFR's of 3:1, 1:1 and 1:3 with the 3:1 and 1:1 MFR's of SnF_2 : AmF 297 yielding the best results. Both SnF_2 and AmF 297 significantly reduced the enamel dissolution rate while AmF 297 also increases the enamel fluoride concentration (Wolf 1964; Shannon 1970, 1971; Shannon and Edmonds 1978; Mühlemann et al. 1957; Mühlemann and Schmid 1958; Mühlemann

and Wolgensinger 1959; Mühlemann 1967; Barbakow, Sener and Imfeld 1984; Barbakow et al. 1987). In contrast to AmF 297, SnF_2 does not increase enamel fluoride concentration (Kirkegaard 1977; Dijkman et al. 1982).

The purpose of the present study was to investigate:

1. The possible synergistic action of the SnF_2 /AmF 297 combination in reducing the enamel dissolution rate. The SnF_2 /AmF 297 combination scheduled for testing included MFR's of 3:1, 1:1 and 1:3. Differences in the loss of phosphorus from the acid-etched treated enamel would indicate the changes in the enamel dissolution rate.

2. Changes in the enamel fluoride concentrations after treating enamel with the SnF_2 /AmF 297 solutions.

In the study, all the SnF_2 /AmF 297 solutions used were aged (at least 4 weeks old) while the SnF_2 solutions were freshly mixed before each topical application.

4. MATERIALS AND METHODS

In this study, 195 surgically extracted human maxillary and mandibular third molars with incomplete root formation were used. The teeth were extracted from patients attending a dental surgery in a non-fluoridated city in the USA and were stored in 0.1% thymol solution at 4°C before use to minimize drying out of the enamel. Each molar was cut into buccal and palatal or mesial and distal halves and the paired specimens were randomly divided for subsequent treatment with the 13 test solutions (Table 1). The specimens were embedded by their roots in acrylic blocks (Paladur, Kulzer Wehrheim, West Germany) to facilitate subsequent immersion of the crowns in their allotted test solutions. In all 780 enamel specimens were prepared and divided among the 6 groups listed in Table 2.

The crowns of the specimens were cleaned using a fluoride-free pumice/distilled water slurry and rotating soft brushes (Rotifix No. 88, Lipert Co., Pforzheim, West Germany) to remove adherent deposits from the enamel, and then stored in humid conditions (0.1% thymol solution) for the duration of the experiment. This prevented the growth of bacteria and fungi on the specimens and minimized enamel brittleness.

The experiment was conducted in two main parts. Specimens in part 1 were immersed in solutions 1 to 9 (Table 1) and subsequently only water-washed after each treatment. In part 2, the specimens were additionally brushed using an abrasive,

fluoride-free dentifrice after each treatment with solutions 10-13 (Table 1). All the enamel specimens were immersed for two minutes in 30 ml of the assigned test solution using a specially prepared apparatus. After the treatments, the specimens were divided into 6 groups and further treated as described below and summarized in Table 2.

Group 1: These enamel specimens were washed for 10 sec using de-ionised water after each treatment to remove excess and unreacted fluoride. The specimens were then stored in the humid 0.1% thymol atmosphere.

Group 2: These enamel specimens were washed for 60 sec using de-ionised water after each treatment and then stored in the humid 0.1% thymol atmosphere.

Group 3: These enamel specimens were washed for 5 min using de-ionised water after each treatment and then stored in the humid 0.1% thymol atmosphere.

Group 4: These enamel specimens were washed for 10 sec using de-ionised water after each treatment and then stored in the humid 0.1% thymol atmosphere.

Group 5: These enamel specimens were brushed with an abrasive, fluoride-free dentifrice for 10 sec after each treatment, then washed for 10 sec using de-ionised water and stored in the humid 0.1% thymol atmosphere.

Group 6: These enamel specimens were brushed with an abrasive, fluoride-free dentifrice for 20 sec after each treatment, then washed for 20 sec using de-ionised water and stored in the humid 0.1% thymol atmosphere.

The specimens in all the groups were immersed in their assigned test solutions twice daily, five days per week, for a 10 week period. In this way each enamel specimen was treated 100 times during the course of the experiment.

At the end of the 10-week immersion period, the crowns were separated from their roots using water-cooled diamond discs (Horico 543/190, Hopf and Ringleb Co., Berlin, West Germany). After separation, the enamel specimens were again washed for 10 sec using distilled water to remove any enamel and dentine dust which was collected on the surfaces.

Fluoride concentrations, amounts of phosphorus and the corresponding depths of etch were determined from each enamel specimen using an in vitro enamel acid-etch technique (van der Merwe et al. 1974). Briefly, two prepared annular self-adhesive discs (3M Co., No. 471, St. Paul, MN., USA), 3 mm in diameter, were placed along the middle third of the crowns (Weatherell, Hallsworth and Robinson 1973). In this way enamel windows were prepared taking care to avoid macroscopic hypoplastic and cracked enamel areas. The rest of the specimen was covered with fluoride-free sticky wax (Klebwachs, Ruscher Co., Zürich, Switzerland), leaving only the two enamel windows exposed. These windows were protected

so that at any one time only one enamel window per specimen was exposed to the acid.

In a blind, complete block design, the enamel windows were acid-etched four times successively in four separate polyethylene beakers each containing 5 ml of 2 N HCl (Merck, Darmstadt, West Germany). The etching times were 5, 10, 15 and 15 sec, respectively, (45 sec; our standard lab. procedure) during which the specimens were regularly agitated.

However, after etching one enamel specimen in each of the 13 treatments, neither phosphorus nor fluoride could be detected in enamel treated with 4 of the 13 test solutions listed in Table 1. These findings were confirmed after etching a second enamel window in each of the 13 treatments. Specimens treated with the identical 4 solutions yielded the same negative results. At this stage the code in the blind study was broken to identify the 4 solutions which rendered the enamel so resistant to a 45 sec etch in 2 N HCl.

A separate study was begun to determine the duration of etch required to dissolve detectable amounts of phosphorus and fluoride from specimens treated with the 4 identified test solutions. From the results of this additional study it was decided to acid-etch all specimens treated with solutions 1 to 13 (Table 1) 8 times successively instead of the usual 4 acid etches. The four additional exposures to acid were set

at 4, 4, 4 and 5 min. Each enamel window in this study was thus finally acid-etched 8 times in 8 separate containers each with 5 ml 2N HCl for a total etching time of 17 min 45 sec (Table 2).

The fluoride concentrations in each of the 8 etching solutions for every enamel window were determined in two 0.5 ml aliquots of the etching solution to which 2 ml of TISAB solution (pH 5.1) was added. Fluoride was determined using a combined reference and fluoride specific ion electrode (906900; Orion Co, USA; Kissa 1983). Standard fluoride curves were only accepted if an angle of 53 to 56 mV was recorded between each decade, particularly between 10^{-5} M F^- and 10^{-6} M F^- . Standard curves were checked every 30 min and re-drawn if necessary. An error of up to 0.5 mV was permitted between the pair of fluoride readings of each etching solution. However, fresh solutions were pipetted if the error between a pair of fluoride readings was greater than 0.5 mV and the new average recorded.

The corresponding phosphorus concentrations in each of the 8 etching solutions per enamel window were determined colorimetrically in two 1 ml aliquots of the etching solutions using the Fiske-Subbarow (1925) method (Hitachi, 100-80 A, Böhringer Mannheim, Rotkreuz, Switzerland). A 5% error between a pair of phosphorus readings for each etching solution was permitted and the average recorded. However, fresh solutions were pipetted if the error between the pair

of phosphorus readings was greater than 5% and that average recorded.

With these results the 8 depths of etch were recorded for the 5 sec, 10 sec, 15 sec, 15 sec, 4 min, 4 min, 4 min, 5 min, respectively. A constant density of enamel of 3 g/ml, a calcium content of 38% and phosphorus content of 18.5% throughout the etched area was assumed (Jenkins, 1978). The depth of etch was calculated using the following formula:

Mass of Dissolved Enamel (μg)

Biopsy Depth (μm) = -----

Density of Enamel X Etched Area (mm^2)

The cumulative fluoride concentrations and the cumulative depths of etch after treatment with the 2 water controls and with the 11 topically applied test solutions were analysed statistically using the One-Way Analysis of Variance Test accepting $P < 0.01$ as being significant (Snedecor and Cochran 1976).

5. SUMMARY OF THE RESULTS

The results of this study are presented in three sections:

(i) The enamel dissolution rates, measured by the amounts of phosphorus dissolved from the 8 etched layers of enamel treated with the 13 test solutions (Table 1).

(ii) The enamel fluoride concentrations in the 8 etched enamel layers treated with the 13 test solutions (Table 1).

(iii) The depths of etch of the 8 enamel layers treated with the 13 test solutions (Table 1).

The results for each of the above three parts are detailed as Tables and bar diagrams. Two bar diagrams were drawn for each series of results; the first bar diagram lists the data obtained for the first four layers etched; the second bar diagram details the results recorded for the 5th to the 8th etched layers.

(i) Enamel Dissolution Rates

The enamel dissolution rates, measured by the amount of phosphorus (μg) dissolved from the 1st etched layer and cumulatively for the subsequently 7 etched enamel layers are listed in Tables 3-8 for the treatment groups 1 to 6, respectively. These results are also shown as bar diagrams in Figs. 1 and 2; 3 and 4; 5 and 6; 7 and 8; 9 and 10; and 11 and 12 for groups 1 to 6, respectively.

(ii) Enamel Fluoride Concentrations

The enamel fluoride concentrations (ppm) in the first etched layer and cumulatively in the subsequently 7 etched enamel layers are listed in Tables 9-14 for the treatment groups 1 to 6, respectively. These results are also shown as bar diagrams in Figs. 13 and 14; 15 and 16; 17 and 18; 19 and 20; 21 and 22; and 23 and 24 for treatment groups 1 to 6, respectively.

(iii) Depths of Etch

The depths (μm) of the first etched enamel layers and the cumulative depths of the subsequently 7 etched enamel layers are listed in Tables 15-20 for the treatment groups 1 to 6, respectively. These results are also shown as bar diagrams in Figs. 25 and 26; 27 and 28; 29 and 30; 31 and 32; 33 and 34; and 35 and 36 for treatment groups 1 to 6, respectively.

6. DETAILED DESCRIPTION OF THE RESULTS

6.1. Enamel dissolution rates

The amount of phosphorus dissolved from the first 4 etched layers differed significantly according to the test solution used. Significantly less phosphorus was dissolved from enamel treated with solutions 2, 3, 4 and 5 (SnF_2/AmF 297 at MFR's of 3:1, 1:1 and 1:3 and SnF_2 , respectively,) compared to treatment with solutions 1, 6, 7, 8 & 9 (water, AmF 297, NaF, Act^R and Candida^R (1500 ppm F). These findings held for the groups that were water-washed for 10 sec (Tables 3 & 6;

Figs. 1 & 7), 60 sec (Table 4; Fig. 3) and 5 min (Table 5; Fig. 5) after their respective treatments. Significantly more phosphorus ($p < 0.001$ or $p < 0.01$) was dissolved from enamel treated with solutions 1, 7, 8 and 9 (water, NaF, Act^R, & Candida^R [1500 ppm F], respectively) than specimens treated with AmF 297 (solution 6). Consequently, the enamel dissolution rate was significantly reduced by the SnF₂-containing test solutions and after treatment with AmF 297.

Significantly less phosphorus ($p < 0.001$) was dissolved from the 5th to the 8th etched enamel layers in the specimens treated with solutions 2, 3, 4, and 5 (SnF₂/AmF 297 at MFR's of 3:1, 1:1, 1:3, and SnF₂ alone respectively), compared to treatment with solutions 1, 6, 7, 8 and 9 (water, AmF 297, NaF, Act^R, & Candida^R [1500 ppm F], respectively). These findings held for the groups that were water-washed for 10 sec (Table 3; Figs. 2 & 8), for 60 sec (Table 4; Fig. 4), and 5 min (Table 5; Fig. 6) after their respective treatments.

Significantly more phosphorus was dissolved from the 5th to the 8th etched layers of the enamel treated with solutions 2, 3 and 4 (SnF₂/AmF 297 at MFR's of 3:1, 1:1, & 1:3, respectively,) compared to treatment with solution 5 (SnF₂).

A trend was observed from Figs. 2, 4 and 6 in that similar quantities of phosphorus, or a similar enamel dissolution rate reduction occurred in enamel treated with solutions 2

(75% SnF_2 & 25% AmF 297) and 3 (50% SnF_2 & 50% AmF 297). The amounts of phosphorus, however, dissolved from enamel treated with solution 4 (25% SnF_2 & 75% AmF 297) were greater in the 4th to 8th etched layers than the corresponding 4 enamel layers of the specimens treated with solutions 2 and 3.

The amounts of phosphorus dissolved in the 8 etched layers after treatment with solutions 10-13 (groups 4-6; water-washed & brushed specimens) are presented in Tables 6-8 and in Figs. 7 and 8; 9 and 10; and 11 and 12; for groups 4 to 6, respectively.

There were no significant differences in the amounts of phosphorus dissolved in the respective 8 layers of the enamel specimens treated with solutions 11, 12 and 13 (Act^{R} , $\text{Candida}^{\text{R}}$ [1500 ppm F] & water, respectively) and then water-washed for 10 sec (group 4; Table 2). However, only minimal amounts of phosphorus were found in the first four etched layers of enamel treated with solution 10 (50% SnF_2 & 50% AmF 297; Table 6 and Fig. 7). This amount of phosphorus was significantly less ($p < 0.001$) compared to the enamel treated with solutions 11, 12, and 13 (Act^{R} , $\text{Candida}^{\text{R}}$ & water, respectively). Also in the 5th to the 8th etched layers, the loss of phosphorus from enamel treated with solution 10 was significantly less ($p < 0.001$) than that of enamel treated with solutions 11, 12 and 13 (Table 6; Fig. 8).

In group 5 (water-washed for 10 sec and brushed for 10 sec after each treatment with solutions 10 to 13; Table 2)

enamel treated with solution 10 (SnF_2/AmF 297; MFR 1:1) had significantly more ($p < 0.001$ or $p < 0.01$) phosphorus dissolved from the first 4 layers than the respective specimens which were only water-washed for 10 sec after each application (Figs. 7 & 9; Tables 6 & 7). Specimens treated with solutions 10 to 12 had significantly less ($p < 0.001$ or $p < 0.01$) phosphorus dissolved than the specimens treated with water (solution 13; Fig. 9; Table 7). However, in the 5th to the 8th etched layers there were no significant differences in the respective amounts of phosphorus dissolved from the enamel after treatment with test solutions 10 to 13 (Table 7; Fig. 10).

In group 6 (water-washed for 20 sec and then brushed for 20 sec after each treatment with solutions 10 to 13; Table 2) enamel treated with solutions 10, 11, and 12 had significantly less ($p < 0.001$ or $p < 0.01$) phosphorus dissolved from the first 4 etched layers than enamel treated with water (solution 13; Fig. 11; Table 8). Significantly less ($p < 0.01$) phosphorus was dissolved from the first 3 layers etched of enamel treated with solution 10 (SnF_2/AmF 297; MFR 1:1) compared with the specimens treated with solution 11 (Act^{R}). But there were, however, no significant differences in the amount of phosphorus dissolved from the first four layers of the specimens treated with solutions 10 and 12 (SnF_2/AmF 297; MFR 1:1 vs $\text{Candida}^{\text{R}}$) although their fluoride concentrations were 250 and 1500 ppm F, respectively.

There were no differences in the amounts of phosphorus dissolved in the 5th to the 8th etched layers of the enamel treated with solutions 10 to 13 (Table 8; Fig. 12).

6.2. Enamel fluoride concentrations

The enamel fluoride concentrations (ppm) in the first etched layer and cumulatively in the seven subsequently etched enamel layers are listed in Tables 9 to 14 for groups 1 to 6, respectively. The results are also shown as bar diagrams in Figs. 13 and 14; 15 and 16; 17 and 18; 19 and 20; 21 and 22; and 23 and 24 for groups 1 to 6, respectively.

In group 1 (water-washed for 10 sec after each treatment) no fluoride was found in the first 4 etched layers of enamel treated with solutions 2, 3 and 5 (SnF_2/AmF 297 at MFR's of 3:1 & 1:1 and SnF_2 , respectively; Table 9; Fig. 13). Minimal amounts of fluoride were found in only the 2nd to the 4th etched enamel layers in the group treated with solution 4 (SnF_2/AmF 297; MFR 1:3). The enamel fluoride concentrations of the first four layers etched of the specimens treated with solutions 6 to 9 were significantly higher ($p < 0.001$) than that of the water control. Exceptions occurred in the first and second etched layers of enamel treated with solution 7 (NaF) where the results were significantly different ($p < 0.05$ & $p < 0.01$, respectively).

In the 2nd, 3rd and 4th etched layers enamel treated with solution 6 (AmF 297) the fluoride concentrations were

significantly higher ($p < 0.001$) than that of enamel treated with solutions 7, 8 and 9 (NaF, Act^R & Candida^R, respectively; Table 9; Fig. 13). However, in the first etched layer the enamel fluoride concentration was similar for specimens treated with AmF 297 and Candida^R (solutions 6 & 9) even though their concentrations were 250 to 1500 ppm F, respectively. Enamel treated with solution 9 (Candida^R, 1500 ppm F) had significantly higher fluoride concentrations in the 2nd, 3rd and 4th etched layers compared to enamel treated with solutions 7 and 8 (NaF & Act^R). In the 5th to 8th etched layers, the enamel fluoride concentration of specimens treated with solutions 2, 3, 4 and 6 (SnF₂/AmF 297 at MFR's of 3:1, 1:1, 1:3, & AmF 297, respectively) were significantly higher ($p < 0.001$ or $p < 0.01$) compared to that of the water control (solution 1; Table 9; Fig. 14).

In group 2 (water washed for 60 sec after each treatment) no or little fluoride was measured in the 1st to the 4th etched layers of enamel treated with solutions 2, 3, 4 and 5 (SnF₂/AmF 297 at MFR's of 3:1, 1:1 & 1:3 and SnF₂, respectively; Table 10; Fig. 15). The fluoride concentrations of the 1st four etched layers of enamel treated with solutions 6 and 9 (AmF 297 & Candida^R) were significantly higher ($p < 0.001$) than that of the water control (solution 1). In the 2nd, 3rd and 4th etched layers of enamel treated with solution 6 (AmF 297) the fluoride concentration was significantly higher ($p < 0.001$) than in the specimens treated with solution 9 (Candida^R).

Fluoride concentrations in the 5th to the 8th etched layers of enamel treated with solutions 2, 3, 4 and 6 (SnF_2/AmF 297 at MFR's of 3:1, 1:1, 1:3 and AmF 297, respectively; Table 10; Fig. 16) were significantly higher ($p < 0.001$ or $p < 0.01$) than that of the water control (solution 1). Enamel treated with solution 5 (SnF_2) had only minimal fluoride concentrations in the 5th to 8th etched layers.

In group 3 (water-washed for 5 min after each treatment) only minimal fluoride concentrations were found in the first four etched layers of enamel treated with solutions 2, 3, 4 and 5 (SnF_2/AmF 297 at MFR's of 3:1, 1:1, 1:3, and SnF_2 , respectively). The fluoride concentrations in the 1st to the 4th etched layers of enamel treated with solutions 6, 7, and 9 (AmF 297, NaF & Candida^R, respectively) were significantly higher ($p < 0.001$) than that of the water control (solution 1; Table 11; Fig. 17).

The fluoride concentrations of enamel treated with solutions 6 and 9 (AmF 297, Candida^R at 1500 ppm F) were significantly higher than that of enamel treated with solution 8 (Act^R, 250 ppm F). The fluoride concentrations in the 5th, 6th, 7th and 8th etched layers were significantly higher ($p < 0.001$ or $p < 0.01$) for enamel treated with solutions 2, 3, 4, 6 and 9 (SnF_2/AmF 297 at MFR's of 3:1, 1:1, 1:3, AmF 297 & Candida^R, respectively) compared to the water control (solution 1). Exceptions to this were the 5th and 6th etched layers of enamel treated with solutions 9 and 2 (Candida^R and SnF_2/AmF

297 at MFR 3:1, respectively. The enamel fluoride concentrations of the specimens treated with solutions 5, 7 and 8 (SnF_2 , NaF & Act^R, respectively) were not significantly increased compared to the water control (Table 11; Fig. 18).

In group 4 (enamel specimens water-washed for 10 sec after each treatment) the fluoride concentrations in the first four layers were significantly higher only after treatment with solution 12 (Candida^R, 1500 ppm F) compared to the water control (solution 13; Table 12; Fig. 19). Although no fluoride was detected in the first and second etched layers treated with solution 10 (SnF_2/AmF 297; MFR 1:1) minimal amounts were found in the third and fourth etched layers. In contrast, the 5th to 8th etched layers enamel treated with solution 10 had significantly more ($p < 0.001$) fluoride compared to the water control (solution 13; Table 12; Fig. 20).

In group 5 (water-washed for 10 sec and brushed for 10 sec after each treatment) the fluoride concentrations of enamel treated with solutions 10, 11 and 12 (SnF_2/AmF 297 at MFR 1:1, Act^R & Candida^R, respectively) were significantly increased ($p < 0.001$) in the first four etched layers compared to the water control (solution 13; Table 13; Fig. 21). The fluoride concentration of enamel treated with solution 12 was significantly higher ($p < 0.001$) compared to enamel treated with solution 10, except in the first etched layer.

The fluoride concentrations of the 5th to 8th etched layers of enamel treated with solutions 10, 11, and 12 (SnF_2/AmF 297 at MFR 1:1, Act^{R} & $\text{Candida}^{\text{R}}$, respectively) were significantly higher ($p < 0.001$) than that of the water control (solution 13; Table 13; Fig. 22). The enamel fluoride concentrations of the specimens treated with solution 12 ($\text{Candida}^{\text{R}}$ 1500 ppm F) were significantly higher ($p < 0.001$) than those of the specimens treated with solutions 10 and 11 (SnF_2/AmF 297 at MFR 1:1 and Act^{R} , respectively).

In group 6 (enamel water-washed for 20 sec and brushed for 20 sec after each treatment) the enamel fluoride concentrations in the 1st to the 4th etched layers of enamel treated with solution 10, 11 & 12 (SnF_2/AmF 297 MFR of 1:1, Act^{R} , & $\text{Candida}^{\text{R}}$, respectively) were significantly higher ($p < 0.001$) than that of the water control group (solution 13; Table 14; Fig. 23). The fluoride concentrations of enamel treated with solution 12 ($\text{Candida}^{\text{R}}$) were significantly increased ($p < 0.001$ or $p < 0.01$) compared to the enamel treated with solution 10, (SnF_2/AmF at MFR 1:1) with the exception of the first etched layer.

The fluoride concentrations of the 5th to the 8th etched layers of enamel treated with solutions 10, 11 & 12 (SnF_2/AmF 297 at MFR 1:1, Act^{R} , & $\text{Candida}^{\text{R}}$, respectively) were significantly increased ($p < 0.001$ or $p < 0.01$) compared to that of the water control group (solution 13). Exceptions to this were for the 5th etched layer of enamel treated with

solutions 10 and 11 (SnF_2/AmF 297 at MFR 1:1 & Act^{R} , respectively. Enamel treated with solution 12 ($\text{Candida}^{\text{R}}$, 1500 ppm F) had significantly higher ($p < 0.001$) fluoride concentrations than that enamel treated with solution 10 (Act^{R} , 250 ppm F; Table 14; Fig. 24).

6.3. Depths of etch

The depths of etch (μm) in the first etched layer and cumulatively in the seven subsequently etched enamel layers are listed in Tables 15 to 20 for groups 1 to 6 (Table 2), respectively. These results are also shown as bar diagrams in Figs. 25 and 26; 27 and 28; 29 and 30; 31 and 32; 33 and 34; and 35 and 36 for groups 1 to 6, respectively.

In group 1 (enamel water-washed for 10 sec after each treatment) there was no or minimal etching of the enamel in the first four layers in the specimens treated with solutions 2, 3, 4 and 5 (SnF_2/AmF 297 at MFR's of 3:1, 1:1, 1:3 and SnF_2 , respectively). Enamel treated with solutions 1, 7, 8 & 9 (water, NaF , Act^{R} 250 ppm F, & $\text{Candida}^{\text{R}}$ 1500 ppm F, respectively) had similar corresponding depths of etch in the first four layers. Specimens treated with solution 6 (AmF 297), however, had significantly lower ($p < 0.001$) depths of etch compared with the water control group (Table 15; Fig. 25). In the 5th to the 8th etched layers the depths of etch of enamel treated with solutions 2, 3, 4 and 5 (SnF_2/AmF 297 at MFR's of 3:1, 1:1 and 1:3, and SnF_2 , respectively) were significantly lower ($p < 0.001$) than that

treated with solutions 1, 6, 7, 8 and 9 (water, AmF 297, NaF, Act^R & Candida^R, respectively; Table 15; Fig. 26).

In group 2 (water-washed for 60 sec after each treatment; Table 16; Fig. 27) no or minimal etching was found for enamel treated with solutions 2, 3, 4 and 5 (SnF₂/AmF 297 at MFR's of 3:1, 1:1, 1:3, and SnF₂, respectively). There were no significant differences in depths of etch between the corresponding first four etched layers of enamel treated with solutions 7, 8 and 9 (NaF, Act^R & Candida^R, respectively) compared to the water control group (solution 1). In the first 4 etched layers, enamel treated with solution 6 (AmF 297) had significantly lower ($p < 0.001$) depths of etch compared to the water control.

In the 5th to 8th etched layers the etched depths of enamel treated with solutions 2, 3, 4 and 5 (SnF₂/AmF 297 at MFR's of 3:1, 1:1, 1:3, and SnF₂, respectively) were significantly lower ($p < 0.001$) than enamel treated with solutions 1, 6, 7, 8 and 9 (water, AmF 297, NaF, Act^R & Candida^R, respectively; Table 16; Fig. 28).

In group 3 (water-washed for 5 min after each treatment) similar depths of etch were found in the first four corresponding enamel layers treated with solutions 2, 3, 4 and 5 (SnF₂/AmF 297 at MFR's of 3:1, 1:1, 1:3, & SnF₂, respectively). There were no significant differences in the depths of the 1st etched enamel layers treated with

solutions 7, 8 and 9 (NaF, Act^R & Candida^R 1500 ppm F, respectively) compared to that of the water control (solution 1). However, enamel treated with solution 6 (AmF 297) was significantly less ($p < 0.001$) etched than the water control group. In the 2nd to the 4th etched layers enamel treated with solutions 6, 7 & 9 (AmF 297, NaF, & Candida^R 1500 ppm F, respectively) were significantly less ($p < 0.001$ or $p < 0.01$) etched than the control (solution 1). However, enamel treated with solution 8 (Act^R) had depths of etch similar to the control (Table 17; Fig. 29). In the 5th to the 8th etched layers, enamel treated with solutions 2, 3, 4 and 5 (SnF₂/AmF 297 at MFR's of 3:1, 1:1, 1:3, & SnF₂, respectively) were significantly less ($p < 0.001$) etched than enamel treated with solutions 1, 6, 7, 8 and 9 (water, AmF 297, NaF, Act^R & Candida^R 1500 ppm F, respectively: Table 17; Fig. 30).

In group 4 (enamel water-washed for 10 sec after each treatment) the first 4 layers of enamel treated with solution 10 (SnF₂/AmF 297 at MFR of 1:1) were significantly less ($p < 0.001$) etched than enamel treated with solutions 11, 12 and 13 (Act^R, Candida^R 1500 ppm F & water, respectively). Except for the 3rd etched layer of enamel treated with solution 12 (Candida^R 1500 ppm F; Table 18; Fig. 31) the respective depths of etch of enamel treated with solutions 11 and 12 (Act^R, Candida^R 1500 ppm F) were similar to that of the control (solution 13). In the 5th to the 8th etched

layers, enamel treated with solution 10 (SnF_2/AmF 297 at MFR of 1:1) was significantly less ($p < 0.001$) etched than enamel treated with solutions 11, 12 and 13 (Act^{R} , $\text{Candida}^{\text{R}}$ 1500 ppm F & water, respectively; Table 18; Fig. 32). There were no significant differences in the respective depths of etch of the specimens treated with solutions 11, 12 and 13 (Act^{R} , $\text{Candida}^{\text{R}}$ and water, respectively).

In group 5 (water-washed for 10 sec and brushed for 10 sec after each treatment) enamel treated with solutions 10, 11 and 12 (SnF_2/AmF 297 at MFR of 1:1, Act^{R} & $\text{Candida}^{\text{R}}$ 1500 ppm F, respectively) were significantly less ($p < 0.001$ or < 0.01) etched in the first four layers than that of the water control group (Table 19; Fig. 33). In the 5th to the 8th etched layers, there were no significant differences in the respective depths of etch for enamel treated with solutions 10, 11, 12 and 13 (SnF_2/AmF 297 at MFR of 1:1, Act^{R} & $\text{Candida}^{\text{R}}$ 1500 ppm F & water, respectively; Table 19; Fig. 34).

In group 6 (water washed for 20 sec and brushed for 20 sec after each treatment) the first four layers of enamel treated with solutions 10, 11 and 12 (SnF_2/AmF 297 at MFR of 1:1, Act^{R} & $\text{Candida}^{\text{R}}$ 1500 ppm F, respectively) were significantly less ($p < 0.001$ or $p < 0.01$) etched than that of the water control group (solution 13; Table 20; Fig. 35). There were no significant differences in the respective depths of etch in 5th to the 8th etched layers of enamel

treated with solution 10 (SnF_2/AmF 297 at MFR of 1:1) compared to the water control group. Occasionally significant differences ($p < 0.01$) in depths of etch were recorded between enamel treated with solutions 11 and 12 (Act^{R} 250 ppm F & $\text{Candida}^{\text{R}}$ 1500 ppm F, respectively) compared to the water control group (Table 20; Fig. 36) but this would not be of any clinical importance.

The conventional profiles of enamel fluoride concentrations to depths of etch for fluoride uptake and retention studies were not included in the results. The reason for this was because the etched depths and the fluoride concentrations of the enamel specimens treated with the SnF_2 -containing test solutions complicated the typical shapes of the profiles. This particularly in the first to the fourth etched enamel layers.

7. DISCUSSION

Under the conditions of the present study the least amounts of phosphorus were dissolved from enamel treated with freshly prepared SnF_2 or with aged, precipitate-free, aqueous solutions of SnF_2/AmF 297. Thus the reductions of the enamel dissolution rates of the above test solutions were significantly better compared to that produced by treatment with either AmF 297, NaF or water. This reduction was shown even though the fluoride concentration of the SnF_2/AmF 297 test solutions was only 250 ppm F. Of the 3 different MFR's of the solutions containing SnF_2 and AmF 297, the 1:3 MFR yielded the poorest reduction of the enamel dissolution rate.

It appeared as though decreasing amounts of phosphorus were dissolved from enamel treated with SnF_2 -containing solutions at lower pH scores. The pH of the freshly mixed SnF_2 alone and the aged, precipitate-free aqueous SnF_2/AmF 297 solutions at MFR's of 3:1, 1:1 and 1:3, respectively were 3.4, 3.6, 4.0 and 4.4. With these increasing pH scores, the protection yielded by the SnF_2 -containing solutions decreased.

No or only minimal amounts of phosphorus were detected in the first four etched enamel layers treated with the SnF_2 -containing solutions. No fluoride at all was found in the first four etched layers of enamel treated with freshly

mixed SnF_2 nor with aged SnF_2/AmF 297 solutions at MFR's of 3:1 or 1:1. The reason for this was because the 45 sec exposure to 2N HCL could not etch the "acid protective" layer that had formed on the treated enamel.

In contrast, minimal fluoride concentrations were found in the enamel treated with the 3:1 SnF_2/AmF 297 solution. Enamel treated with AmF 297 (250 ppm F; soln. 6) had a significantly higher fluoride concentration than enamel treated with the other test solutions, except those treated with solution 9 which had a fluoride content of 1500 ppm F. When applying topical solutions of low fluoride concentration to enamel, only minimal increases in the enamel fluoride levels can be expected. The possible influence of the initial fluoride concentration of the enamel specimens on the subsequent fluoride retention in enamel, both in vitro or in vivo, remains uncertain (Nicholson and Mellberg 1969; Heifetz et al. 1970).

The third molars used in this study were obtained from a dental surgery in a non-fluoridated city but their individual pre-extraction fluoride histories were unknown. Because the third molars were either impacted or partially impacted before extraction, they were unlikely to have been affected by any post-eruptive uptake from dilute or concentrated topical fluoride agents. Summerlin, Retief and Denton (1984) reported enamel fluoride concentrations varying from

334 to 3868 ppm F on different areas in the same molar teeth.

Enamel dissolution rate changes and enamel fluoride concentrations can be assessed by acid-etch, abrasive or chemical methods (Mühlemann, Schait and König 1964; Weatherell and Hargreaves 1965; Brudevold, McCann and Gron 1968; Aasenden, Moreno and Brudevold 1972; Baijots-Stroobants 1980; Uchtmann and Duschner 1982; Apap and Goldberg 1985; Barbakow et al. 1987). These methods all have inherent advantages and disadvantages.

The detailed direction of etch in the acid-etch technique can not be controlled nor can the etched depth be standardised. However, in this study the actual depth of etch was recorded, and a statistically standardised depth as described by Retief, Harris and Bradley (1985) was not considered acceptable. The inability to control the depth of etch is a major disadvantage particularly when using SnF_2 -containing solutions, as the present results showed. Under the conditions of this present study, in vitro SnF_2 treatment apparently formed a deposit on enamel which could resist the etching effect of even 2 N HCl for 45 sec. The in vitro enamel dissolution rate was massively reduced. Under similar experimental conditions, neither NaF nor AmF 297 (250 ppm F) reduced the enamel dissolution.

The reproducibility of the in vitro acid-etch technique was shown by comparing the present results of the water control group with similar groups in other related experiments carried out in our laboratories (Barbakow et al. 1987). A criticism of the present method is the lack of simulating intra-oral conditions closely enough. Precipitates formed on fluoride-treated enamel can be removed by washing the enamel in various types of artificial salivas (Retief et al. 1983) for different time periods. Further, precipitate formation can be reduced or inhibited by treating enamel with organic solutions similar to that found intra-orally.

Agents that produce a decreased enamel solubility, effective inhibition of plaque growth and/or reduced caries-promoting bacteria in vitro may fail to achieve these results in clinical tests. Failure to do so could be due to several factors. These include non-predictive laboratory tests, or incorrectly chosen concentrations of active agents and their duration of activity intra-orally. Deactivation of the agent by contact with saliva and/or plaque must also be considered.

Phosphorus dissolved from the first four enamel layers after treatment with aged SnF_2/AmF 297 at MFR's of 3:1, 1:1 and 1:3 or with fresh SnF_2 and then water-washed for 10 sec, 60 sec and 5 min was significantly less than enamel similarly treated with water, AmF 297, NaF, Act^R, (250 ppm F) or

Candida^R (1500 ppm F). Specimens treated with AmF 297 had significantly less phosphorus dissolved from the enamel in groups 1, 2 and 3 than the other non-SnF₂-containing solutions. However, brushing enamel after treatment with the 4 test solutions (10-13) removed the "acid protective" layer. This resulted in similar amounts of phosphorus being dissolved from enamel treated with aged SnF₂/AmF 297, Act^R, Candida^R, and water. The 10- and 20-sec in vitro brushing of the treated specimens was a massive challenge and it is unlikely that individual teeth in vivo are ever brushed for such long periods of time nor at the pressures used in the study.

Fluoride-containing mouth rinses have been tested mainly using NaF and SnF₂ (Volpe 1982; Mellberg and Ripa 1983). The advantages of NaF in a rinse include its good solubility in water, its neutral taste and remineralising potential of initial enamel white spot lesions. In contrast, SnF₂ is poorly soluble and not stable in water, has a pungent taste and although it remineralises initial enamel lesions well, they are frequently stained light brown to black. However, the SnF₂/AmF 297 mouth rinse clinically reduced plaque growth, sulcus bleeding index and sulcus fluid flow (Mühlemann, Saxer and Mörmann 1980; Mühlemann 1981 & 1984). No exogenous dental stains nor taste disturbances were reported after using the SnF₂/AmF 297 mouth rinse. Such

complications occur frequently when using chlorhexidine and other potent antibacterial mouth rinses.

Both SnF_2 and AmF 297 reduce enamel dissolution rate and facilitate the remineralization of initial enamel carious lesions (Hals, Torell and Mörch 1960; Mühlemann, 1967; Shannon and Feller 1973; Schneider and Mühlemann 1974; Hock and Tinanoff 1979; Tinanoff and Weeks 1979; Baijot-Stroobants and Vreven 1980). However, no synergistic effect of the SnF_2 and the AmF 297 was proved in the present study. On the contrary, increasing the amount of AmF 297 negatively influenced the potential of the SnF_2 to reduce the enamel dissolution rate. Nelson (1978) reported that increasing the amount of stannous ion in a SnF_2 system enhanced the formation of $\text{SnF}_3\text{F}_3\text{PO}_4$. The ideal in that study was to have a solution containing stannous and fluoride ions in a 1:1 ratio. That finding was partially confirmed by the results of the present study. Increasing the temperature of 8% SnF_2 solutions from 25°C, 45°C, 65°C to 85°C increased the in vitro enamel fluoride uptake in both normal and etched enamel (Putt, Beltz and Muhler 1978). In the present study, however, the enamel was treated with 0.1% SnF_2 at room temperature.

Most studies on increases of enamel fluoride concentrations or on reductions of enamel dissolution rates are carried out using concentrated amounts of fluoride (Mellberg et al.

1966; Retief et al. 1983; de Bruyn et al. 1985; Goorhuis and Purdell-Lewis 1986). Few studies have reported the effects after applying dilute fluoride concentrations (Mühlemann, Schait and König 1968; Mellberg et al. 1973; Shannon 1980). In the present study, the fluoride concentration was up to 50 times lower than that generally tested. In 1966 Mellberg et al. showed that most of the acquired fluoride from concentrated topical agents was lost within the first 24 h by washing in water. Because of the lowered fluoride concentrations tested in the present study, short washing periods (10 sec, 60 sec & 5 min) were selected. Further, the treated enamel was washed in fast-flowing water, instead of simply immersing the specimens in water, following each topical treatment.

Difficulties arise when comparing other studies' results of enamel dissolution rate changes after topical fluoridation because of variations in the experimental methods employed. For example etching times, types of acid and their normalities differ. The duration of the in vitro treatments differ and the frequency of treatment varies from one to 50 applications. The treated enamel can subsequently be washed for varying periods of time in either water, KOH or various types of artificial saliva (Dijkman et al. 1982; Wei and Connor 1983; Wefel and Harless 1984) and finally Stearns (1972) questioned the effect of stannous ions generally on spectrophotometric phosphorus determinations.

Examples below illustrate how the methods of the present study differ from that of other related investigations. Dijkman et al. (1982) immersed enamel only once in 4% SnF_2 for 5 or 30 mins, washed the specimens in 1 M KOH for 24 h and then acid etched the enamel in 0.1 M HClO_4 (0.5 ml) for 30, 30, 60, 120 & 120 sec. Immersion in KOH caused the control and the SnF_2 -treated specimens to have similar enamel dissolution rates irrespective of the immersion time in the SnF_2 . This indicates that the KOH dissolved the "acid-protective" layer from the treated enamel. Therefore the 24 h immersion in KOH removed the "protective" effects of the SnF_2 on enamel.

In contrast, Shannon (1980) showed a significantly lowered enamel dissolution rate with SnF_2 compared to NaF solutions containing 900 and 225 ppm F after a 2 min treatment and subsequent dissolution in 0.025 M lactic acid. Unfortunately, in that study neither the washing nor the etching procedures were listed. Kirkegaard (1977) immersed enamel once in 8% SnF_2 , washed the specimens in water for 30 sec and etched them using 0.5 M HClO_4 three times successively for 15 sec each and then twice more for 30 sec each. In each respective layer, the amount of fluoride in enamel was not different compared to NaF but the amount of enamel etched was less. However, the present study showed that tooth brushing immediately after immersion in SnF_2/AmF

297 at MFR of 1:1 also partially removed the "acid protective" layer formed on the enamel.

Shannon (1969) found that 72% of the stannous ions were lost after 15 months in an aqueous 0.4% SnF_2 solution compared to no significant loss of stannous ions from a 0.4% non-aqueous SnF_2 solution in glycerine. Shannon and Edmonds (1979) reported that enamel treated with 10%, 0.4% or 0.1% SnF_2 solutions became increasingly more resistant to acid etching with increasing time (15 sec, 30 sec, 1 min, 2 min & 4 min). However, after 1 min treatment there was no significant difference in the effect of the concentration on the amount of phosphorus dissolved from the treated enamel. In the present study the enamel was immersed for 2 min, again because of the lowered fluoride concentration of the test solutions (250 ppm F).

"In selecting the most effective mouthrinse preparations, serious consideration should be given to SnF_2 -containing materials" (Shannon 1980). Because of its proven dental properties, SnF_2 has also been tested in combination with concentrated APF (acidulated phosphofluoride; Shannon, 1970; Shannon, Edmonds and Madsen, 1974). Recently Crall et al. (1983), and Crall and Bjerga (1984) reported the changes on enamel following topical treatment with SnF_2 and APF mixed or sequentially applied in two-step procedures. Enamel fluoride uptake was dependent on the SnF_2 concentration.

However, this finding was not confirmed by the present study.

The nature of the "acid protective" layer formed by SnF_2 on enamel has been examined. Berndt (1972) and Wei and Forbes (1974) reported that $\text{Sn}_3\text{F}_3\text{PO}_4$ and CaF_2 formed on enamel treated with SnF_2 . However, initial SEM studies showed no detectable deposit or precipitate on enamel treated for 5 min with SnF_2 (Gray et al. 1958). Subsequently, Krutchkoff et al. (1972), Wei (1974) and Dijkman et al. (1982) showed that the quantity of the reaction products on enamel increased with increasing exposure time to SnF_2 . On acid-etched surfaces, in contrast, refractile crystals were observed after 4- and 8-min applications of 10% SnF_2 (Nordquist, Krutchkoff and Wei, 1977). Wei (1975) also reported the presence of an amorphous material, which was difficult to resolve under high power, on pre-etched enamel after applying 10 % SnF_2 for 4 min. No surface changes, however, were seen in that study after a 2-min SnF_2 application on the pre-etched enamel. Dijkman et al. (1982) described the presence of a thin layer of globular-shaped products on the enamel after a 5-min application of 4% SnF_2 and subsequent washing for 30 sec. A thicker layer, however, was present when the application time was extended to 30 min. SnF_2 topically applied to enamel following an APF application produced a thicker precipitate on the enamel than when the APF was applied alone, and this precipitate

was not removed during a 24 hour washing (Crall et al. 1982).

Under the conditions of the present in vitro study precipitate-free SnF_2/AmF 297 solutions significantly reduced the amount of phosphorus dissolved from the enamel, or decreased the enamel dissolution rate. Brushing the enamel after treatment with SnF_2/AmF 297 solutions partially removed the protective effects to acid-etching. However, significantly less phosphorus was dissolved from the brushed specimens compared to the control group. The results of this study compared well with earlier findings by Shannon (1980) who has stated that "in selecting the most effective mouth rinse preparations, serious consideration should be given to SnF_2 -containing materials".

8. CONCLUSIONS

1. It was necessary to acid-etch eight enamel layers instead of the planned four layers, because all the SnF_2 -containing test solutions rendered the enamel so resistant to 2 N HCl. In subsequent studies with the SnF_2 /AmF 297 combination, enamel dissolution should be tested using longer water-washing times and other washing solutions (e.g. KOH). In addition, in vivo conditions could be better simulated by firstly immersing the enamel in saliva or other natural or synthetic protein solutions prior to topical treatments.
2. As in previous studies by other researchers, the SnF_2 -containing solutions did not increase the enamel fluoride concentrations.
3. Of all the test solutions only those containing SnF_2 significantly reduced the enamel dissolution rate when the specimens were only water-washed for up to 5 min after each treatment.
4. The best reductions of enamel dissolution rates occurred after treatment with SnF_2 or with SnF_2 mixed with AmF 297.
5. The least reduction of the enamel solution rate, using a SnF_2 -containing solution, was found in enamel treated with the SnF_2 /AmF 297 at MFR of 1:3.

6. The enamel dissolution rates recorded by the SnF_2/AmF 297 solutions at MFR's of 3:1 and 1:1 were almost similar.

7. There was no synergistic reduction of the enamel dissolution rate when combining SnF_2 with the AmF 297.

8. Under the conditions of this study, the enamel dissolution rate was significantly better reduced by the SnF_2/AmF 297 solutions (250 ppm F) at the 3 MFR's than either Act^R or Candida^R containing NaF at 250 or 1500 ppm F, respectively.

9. Brushing the treated enamel for 10 or 20 sec after each treatment with SnF_2/AmF 297 (MFR 1:1) reduced the "protective effect" of the solution but the enamel dissolution rate was still better reduced than by the other test solutions. This indicates that such a mouth rinse should be used after tooth brushing.

10. It appeared as though the amounts of phosphorus dissolved from the enamel decreased with decreasing pH scores of the SnF_2 -containing solutions.

9. SUMMARY

Human enamel specimens were immersed in vitro in 1 of 3 aged, precipitate-free aqueous SnF_2/AmF 297 solutions (250 ppm F) at molar fluoride ratios of 3:1, 1:1 and 1:3, respectively. These results were compared to enamel similarly treated with freshly prepared SnF_2 (positive control), NaF, AmF 297 or water (negative control). All solutions contained 250 ppm F, except one NaF solution which had 1500 ppm F. The specimens were immersed 100 times over 10 weeks (twice daily) in the test solutions, water washed for 10 sec, 60 sec or 5 min, with or without subsequent tooth brushing. Each specimen was successively acid-etched 8 times in 2N HCl for 5 sec, 10 sec, 15 sec, 15 sec, 4 min, 4 min, 4 min, and 5 min (8 times, total 17 min 45 sec). The average enamel dissolution rates, fluoride concentrations and depths of etch were determined for each solution tested and statistically analysed. Enamel treated with SnF_2 and SnF_2/AmF 297 were more resistant ($P < 0.001$) than the other test solutions. However, brushing the specimens removed the protective effects of the SnF_2/AmF 297 solutions. No synergism of the SnF_2 and the AmF 297 was shown concerning the enamel dissolution rate nor for the enamel fluoride concentration. As in other studies the enamel fluoride concentration was not increased by SnF_2 alone nor in combination with the AmF 297.

10. ZUSAMMENFASSUNG

Menschliche Schmelzproben wurden in vitro eingetaucht in 1 von 3 gealterten, präzipitatfreien, wässrigen $\text{SnF}_2/\text{AmF 297}$ -Lösungen (250 ppm F) bei molaren Fluorverhältnissen von 3:1, 1:1 und 1:3. Diese Resultate wurden verglichen mit Schmelzproben, die auf dieselbe Art und Weise behandelt wurden mit frisch präparierter SnF_2 -Lösung (positive Kontrolle) und mit NaF, AmF 297 oder Wasser (negative Kontrolle). Alle Lösungen enthielten 250 ppm F, mit Ausnahme einer NaF-Lösung, welche 1500 ppm F hatte. Die Schmelzproben wurden während 10 Wochen 100 mal (2 mal pro Tag) in die Testlösungen eingetaucht und nach diesen Behandlungen jeweils für 10 Sekunden, 60 Sekunden oder 5 Minuten in Wasser gewaschen, letzteres mit oder ohne darauffolgendes Zähnebürsten. Darauf wurde jede Schmelzprobe nacheinander 8 mal mit 2 N HCl angeätzt für 5 Sek., 10 Sek., 15 Sek., 15 Sek., 4 Min., 4 Min., 4 Min. und 5 Min. (total 17 Min. 45 Sek.). Die durchschnittlichen Schmelzauflösungsraten, die Fluorid-Konzentrationen und die Ätztiefen wurden für jede Testlösung bestimmt und statistisch analysiert. Schmelz, welcher mit SnF_2 und $\text{SnF}_2/\text{AmF 297}$ behandelt wurde, war widerstandsfähiger ($p < 0.001$) gegen Auflösung als Schmelz, der in den anderen Lösungen getaucht wurde. Das Bürsten der Schmelzproben aber entfernte zum Teil die "Schutzeffekte" der $\text{SnF}_2/\text{AmF 297}$ -Lösungen. Kein synergistischer Effekt von SnF_2 und AmF 297 konnte nachgewiesen werden, weder bezüglich Schmelzauflösungsrate noch für die Fluorid-Konzentration.

Wie auch in anderen Studien bestätigt, wurde letztere nicht erhöht durch SnF_2 allein oder in Kombination mit AmF 297.

11. RESUME

Des échantillons d'émail humain ont été immergés in vitro dans une des trois solutions aqueuses vieilles et sans précipité, de SnF_2/AmF 297 (250 ppm F) dans des proportions molaires de fluorures de 3:1, 1:1 et 1:3 respectivement. Ces résultats ont été comparés à ceux obtenus en traitant l'émail dans des conditions identiques avec des solutions fraîches de SnF_2 (contrôle positif), NaF, AmF 297 et d'eau (contrôle négatif). Toutes les solutions contenaient 250 ppm F, excepté une de NaF qui contenait 1500 ppm F. Les exemplaires ont été trempés 100 fois durant les 10 semaines d'expérimentation à raison de deux immersions par jour dans les solutions à tester, puis rinçés à l'eau pendant 10 sec., 60 sec. ou 5 minutes, ces derniers avec ou sans brossage successif des dents. Chaque spécimen a été ensuite mordancé 8 fois dans HCl 2N pendant 5 sec., 10 sec., 15 sec., 15 sec., 4 min., 4 min., 4 min. et 5 min. (total 17 min. 45 sec.). Le degré moyen de dissolution, la concentration en fluorure et la profondeur du mordantage ont été déterminés pour chaque solution à tester et analysés statistiquement. L'émail, traité avec SnF_2 et SnF_2/AmF 297 était plus résistant ($p < 0.001$) que traité avec d'autres solutions. Le brossage des échantillons a complètement supprimé l'effet protecteur des solutions de SnF_2/AmF 297. Aucun synergisme n'a été démontré entre SnF_2 et AmF 297 concernant la dissolution de l'émail et la concentration de celui-ci en fluorure. Confirmant les résultats obtenus dans d'autres

études cette dernière ne fut pas augmentée par SnF_2 seul ou en combinaison avec AmF 297.

12. REFERENCES

- Aasenden, R., Moreno, E. C. and Brudevold, F.: Control of sampling areas for human tooth enamel biopsies. Arch oral Biol 17: 355-358, 1972.
- Apap, M. and Goldberg, M.: A new microsample grinding technique for quantitative determination of calcium and phosphorus in dental enamel. J Dent Res 64: 1293-1295, 1985.
- Arnold, F. A., Likins, R. C., Russel, A. L. and Scott, D. B.: Fifteenth year of the Grand Rapids fluoridation study. J Am Dent Assoc 65: 780-785, 1962.
- Baijot-Stroobants, J. and Vreven, J.: In vivo uptake of topically applied fluoride by human dental enamel. Arch oral Biol 25: 617-621, 1980.
- Barbakow, F., Sener, B. and Imfeld, T.: In vitro fluoride uptake by human enamel after brushing with an amine fluoride gel and subsequent loss of fluoride by water-washing. Helv Odont Acta 28: 28-36, 1984; In Schweiz Mschr Zahnmed 94: 1275-1283, 1984.
- Barbakow, F., Sener, B., Lutz, F. and Imfeld, T.: Fluoride retention by human enamel after in vitro application of nicomethanol hydrofluoride. J Dent Child 54: 9-14, 1987.
- Beiswanger, B. B., Mercer, V. H., Billings, R. J. and Stookey, G. K.: A clinical caries evaluation of a stannous fluoride prophylactic paste and topical solution. J Dent Res 59: 1386-1391, 1980.
- Berndt, A. F.: Reaction of stannous fluoride with hydroxyapatite: the crystal structure of $\text{Sn}_3\text{PO}_4\text{F}_3$. J Dent Res 51: 53-57, 1972.
- Bibby, B.G., Zander, H. A., McKelleget, M., and Labunsky, B.: Preliminary reports on the effect of dental caries of the use of sodium fluoride in a prophylactic cleaning mixture and in a mouthwash. J Dent Res 25: 207-211, 1946.
- Bramstedt, F. and Bandilla, J.: Ueber den Einfluss organischer Fluorverbindungen auf Säurebildung u. Polysaccharidsynthese von Plaques-Streptokokken. Dtsch zahnärztl Z 21: 1390-1396, 1966.

Breitenmoser, Th.: The antiglycolitic action on dental plaque of amine chlorides. *Helv Odont Acta* 19:13-17, 1975.

Brudevold, F., McCann, H. G. and Gron, P.: An enamel biopsy method for determination of fluoride in human teeth. *Arch oral Biol* 13: 877-885, 1968.

Candeli, A., Marci, F. and Fornari, M. T.: Studio "in vitro" sulla solubilizzazione del calcio e del fosforo di denti e di campioni di dentina trattati con diversi dentifrici fluorati. *Riv Ital Stomatol* 22: 627-640, 1967.

CDT ADA: Fluoride Compounds. In Accepted Dental Therapeutics 40th ed., pp.395-420, 1984.

Crall, J. J., Silverstone, L. M., Clarkson, B. H., Wefel, J. S. and Wei, S. H. Y.: Fluoride uptake and in vitro caries-like lesion formation in enamel after two-step topical fluoride applications. *Caries Res* 16: 162-169, 1982.

Crall, J. J., Wefel, J. S., Clarkson, B. H., Silverstone, L. M. and Wei, S. H. Y.: SEM and electron microprobe analysis of enamel treated with two-step topical fluorides in vitro. *Caries Res* 17: 481-489, 1983.

Crall, J. J. and Bjerga, J. M.: Fluoride uptake and retention following combined applications of APF and stannous fluoride in vitro. *Pediatr Dent* 6: 226-229, 1984.

Dean, H. T.: Epidemiological studies in the United States. In Dental Caries and Fluorine, ed. F. R. Moulton, pp 5-31. Washington DC., Amer Assoc Adv of Sci. 1946.

de Bruyn, H., Hummel, M. and Arends, J.: In vivo effect of a fluoridating varnish with various fluoride contents on human enamel. *Caries Res* 19: 407-413, 1985.

DePaola, P. F., Soparkar, P., Foley, S., Bookstein, F. and Bakhos, Y.: Effect of high-concentration ammonium and sodium fluoride rinses on dental caries in schoolchildren. *Community Dent Oral Epidemiol* 5: 7-14, 1977.

Dijkman, A. G., Tak, J., Smith, M. L., Jongebloed, W. L. and Arends, J.: Fluoride deposited by topical applications with stannous fluoride in human enamel. *Jour Biol Buccale* 10: 63-71, 1982.

Fiske, C. H. and Subbarow, Y.: The colorimetric determination of phosphorus. *J Biol Chem* 66: 375-400, 1925.

Fjaestad, M., Norstedt, K. and Torell, P.: Forsok med enkla metoder for klinisk fluorapplikation. *Sverige Tandlak-Forb Tidn* 53: 169-180, 1961.

Gillings, B. R. D., Broadhurst, G. G. and Martin, N. D.: Effect of fluoride-containing dentifrices on tooth enamel solubility. *Aust Dent J* 9: 414-418, 1964.

Gish, C. W., Howell, C. L., and Muhler, J. C.: A new approach to the topical application of fluorides for the reduction of dental caries in children. A preliminary report. *J Dent Res* 36: 784-786, 1957.

Goorhuis, J. and Purdell-Lewis, D. J.: 0.25% and 0.4% amine fluoride gel for weekly topical application. *Caries Res* 20: 458-464, 1986.

Gray, J. A., Schweizer, H. C., Rosevear, F. B. and Broge, R. W.: Electron microscopic observations of the differences in the effects of stannous fluoride and sodium fluoride on dental enamel. *J Dent Res* 37: 638-648, 1958.

Gülzow, H.-J.: Ueber die Beeinflussung der Säurelöslichkeit der Schmelzoberfläche durch Aminfluoride. *Dtsch zahnärztl Z* 21: 290-295, 1966.

Hals, E., Torell, P. and Mörch, T.: Effect of topically applied agents on enamel. V. Experiments in vitro with tin fluoride solutions. *Acta Odont Scand* 18: 455-460, 1960.

Heifetz, S. B., Mellberg, J. R., Winter, S. J. and Doyle, J.: In vivo fluoride uptake by enamel of teeth of human adults from various topical fluoride procedures. *Arch oral Biol* 15: 1171-1181, 1970.

Heifetz, S. B., Driscoll, W. S. and Creighton, W. E.: The effect on dental caries of weekly rinsing with a neutral sodium fluoride or an acidulated phosphate-fluoride mouthwash. J Am Dent Assoc 87: 364-368, 1973.

Hermann, U. and Mühlemann, H. R.: Inhibition of salivary respiration and glycolysis by an organic fluoride. Helv Odont Acta 2: 28-33, 1958.

Hock, J. and Tinanoff, N.: Resolution of gingivitis in dogs following topical applications of 0.4% stannous fluoride and toothbrushing. J Dent Res 58:1652-1653, 1979.

Jenkins, G. N.: The Physiology and Biochemistry of the Mouth. 4th ed. Blackwell Scientific Publications, Oxford. pp. 54-59, 1978.

Kirkegaard, E.: In vitro fluoride uptake in human dental enamel from various fluoride solutions. Caries Res 11: 16-23, 1977.

Kissa, E.: Determination of fluoride of low concentration with the ion-selective electrode. Ann Chem 55: 1445-1448, 1983.

König, K. G.: Dental caries and plaque accumulation in rats treated with stannous fluoride and penicillin. Helv Odont Acta 3: 39-44, 1959.

Krutchkoff, D. J., Jordan, T. H., Wei, S. H. Y. and Nordquist, W. D.: Surface characterization of the stannous fluoride-enamel interaction. Arch oral Biol 17: 923-930, 1972.

Lilienthal, B.: Inhibition of acid formation from carbohydrates by stannous fluoride and stannous chlorofluoride. Aust Dent J 1:165-173, 1956a.

Lilienthal, B.: The effect of a stannous fluoride mouthwash on acid formation in the mouth and some observations on the mechanism of inhibition. Aust Dent J 1:221-227, 1956b.

Lim, J. K. J.: Precipitate-free dilute aqueous solutions of stannous fluoride for topical application. Simple and mixed mediums. J Dent Res 49: 760-767, 1970.

McCormick, J.: A critical review of the literature on mouth-washes. Ann N Y Acad Sci 153: 374-385, 1968.

Mellberg, J. R., Laakso, P. V. and Nicholson, C. R.: The acquisition and loss of fluoride by topically fluoridated human tooth enamel. Arch oral Biol 11: 1213-1220, 1966.

Mellberg, J. R., Nicholson, C. R., Packer, M. W. and Laswell, H. R.: Fluoride concentrations in deciduous teeth of children using fluoride mouthrinses. Caries Res 7: 324-331, 1973.

Mellberg, J. R. and Ripa, L. W.: Self-applied topical fluorides. In Fluoride in Preventive Dentistry; Theory and Clinical Applications. Chicago, Quintessence Publ., pp. 243-277, 1983.

Mühlemann, H. R., Schmid, H. and König, K. G.: Enamel solubility reduction studies with inorganic and organic fluorides. Helv Odont Acta 1: 23-33, 1957.

Mühlemann, H. R. and Schmid, H.: Anticaries dentifrices under laboratory conditions. J Dent Belge 49: 353-372, 1958.

Mühlemann, H. R. and Wolgensinger, F.: In vivo reduction of enamel solubility in children using an organic fluoride dentifrice. Helv Odont Acta 3: 35-39, 1959.

Mühlemann, H. R., Schait, A. and König, K. G.: A chemical method for the removal of enamel surface layers and its suitability for fluoride analysis. Helv Odont Acta 8: 147-153, 1964.

Mühlemann, H. R.: Die kariesprophylaktische Wirkung der Aminfluoride - 10 Jahre Erfahrungen. Quintessenz 18: 113-120, May; 123-127, June; 109-119, July; 109-117, August; 1967.

Mühlemann, H. R., Schait, A. and König, K. G.: Fluorine uptake of enamel and animal caries inhibition by topical sodium fluoride and amine-fluorides at low fluoride concentrations. Helv Odont Acta 12: 61-66, 1968.

Mühlemann, H. R. and Rudolf, E. R.: Fluoride retention after rinsing with sodium fluoride and amine fluoride. *Helv Odont Acta* 19: 81-84, 1975.

Mühlemann, H. R., Saxer, U. P. and Mörmann, W.: Reduction of plaque and gingivitis by stannous fluoride stabilized with amine fluoride. ORCA congress, Marburg, Abstract No. 20, 1980.

Mühlemann, H. R.: Auf dem Weg zum sauberen Zahn? *Swiss Dent* 2: 7-9, 1981.

Mühlemann, H. R.: 25 Jahre Schweizer Plaque-Pharmakologie. *Schweiz Mschr Zahnmed* 94: 91-101, 1984.

Mühlemann, H. R. and Schmid, H.: Orale Kompositionen mit stabilisierten Zinnsalzen. *Europ, Pat. No. 0 026 539*, Owner: Gaba Int Ag, Basel, 1985.

Muhler, J. C. and Van Huysen, G.: Solubility of enamel protected by sodium fluoride and other compounds. *J Dent Res* 26: 119-127, 1947.

Muhler, J. C. and Day, H. G.: Effects of stannous fluoride, stannous chloride and sodium fluoride on the incidence of dental lesions in rats fed a caries-producing diet. *J Am Dent Assoc* 41: 528-535, 1950.

Muhler, J. C., Nebergall, W. H., and Day, H. G.: Preparations of stannous fluoride compared with sodium fluoride for the prevention of dental caries in the rat. *J Am Dent Assoc* 46: 290-295, 1953.

Muhler, J. C., Radike, A. W., Nebergall, W. H., and Day, H. G.: A comparison between the anti-cariogenic effect of dentifrices containing stannous fluoride and sodium fluoride. *J Am Dent Assoc* 51: 556-559, 1955.

Muhler, J. C., Radike, A. W., Nebergall, W. H. and Day, H. G.: The effect of a stannous fluoride-containing dentifrice on dental caries in adults. *J Dent Res* 35: 49-53, 1956.

Muhler, J. C.: The effect of a single topical application of stannous fluoride on the incidence of dental caries in adults. *J Dent Res* 37: 415-416, 1958.

Nelson, K. G.: Rate of stannous fluoride reaction with hydroxyapatite. *J Dent Res* 57: 162-165, 1978.

Nicholson, C. R. and Mellberg, J. R.: Effect of natural fluoride concentration of human tooth enamel on fluoride uptake in vitro. *J Dent Res* 48: 302-306, 1969.

Nordquist, W. D., Krutchkoff, D. J. and Wei, S. H. Y.: Influence of variable SnF_2 exposure time on in vitro $\text{Sn}_3\text{F}_3\text{PO}_4$ formation. *Caries Res* 11: 39-45, 1977.

Pohto, P., Antila, R. and Toivanen, E.: Comparison of fluorine-containing dentifrices under laboratory conditions. *Suom Hammaslääk Toim* 67: 189-198, 1971.

Putt, M. S., Beltz, J. F. and Muhler, J. C.: Effect of temperature of SnF_2 solution on tin and fluoride uptake by bovine enamel. *J Dent Res* 57: 772-776, 1978.

Retief, D. H., Bradley, E. L., Holbrook, M. and Switzer, P.: Enamel fluoride uptake, distribution and retention from topical fluoride agents. *Caries Res* 17: 44-51, 1983.

Retief, D. H., Harris, B. E. and Bradley, E. L.: In vitro enamel fluoride uptake from topical agents. *Dent Material* 1: 93-97, 1985.

Ringelberg, M. L., Webster, D. B., Dixon, D. O. and LeZotte D. C.: The caries-preventive effect of amine fluorides and inorganic fluorides in a mouthrinse or dentifrice after 30 months of use. *J Am Dent Assoc* 98: 202-208, 1979.

Roberts, J. F., Bibby, B. G., and Wellock, W. D.: The effect of an acidulated fluoride mouthwash on dental caries. *J Dent Res* 27: 497-500, 1948.

Schneider, P. and Mühlemann, H. R.: The antiglycolytic action of amine fluorides on dental plaque. *Helv Odont Acta*, 18 (Suppl. VIII): 63-70, April, 1974.

Shannon, I. L.: Water-free solutions of stannous fluoride and their incorporation into a gel for topical application. Caries Res 3: 339-347, 1969.

Shannon, I. L.: Preventive dental services in the Veterans' Administration Hospital. J Pub Health Dent 30: 156-162, 1970.

Shannon, I. L.: In vitro enamel solubility reduction through sequential application of acidulated phosphofluoride and stannous fluoride. J Canad Dent Assn 8: 308-310, 1970.

Shannon, I. L.: Antisolubility effects of acidulated phosphofluoride and stannous fluoride in the treatment of crown and root-surfaces. Austr Dent J 16: 240-242, 1971.

Shannon, I. L. and Feller, R. P.: Effect of extended storage on aqueous solutions of stannous fluoride. J Indiana Dent Assoc 45: 59-64, 1973.

Shannon, I. L., Edmonds, E. J. and Madsen, K. O.: Single, double, and sequential methods for fluoride applications. J Dent Child 41: 35-38, 1974.

Shannon, I. L. and Edmonds, E. J.: Solubility reduction of enamel, dentin and root surfaces by NaF and SnF_2 . J Missouri Dent Assn 58: 16-23, 1978.

Shannon, I. L. and Edmonds, E. J.: Topical applications of stannous fluoride: Choice of concentration and duration of treatment. J Dent 7: 9-14, 1979.

Shannon, I. L. and West, D. C.: Prevention of decalcification in orthodontic patients by daily self-treatment with 0.4% SnF_2 gel. Pediatr Dent 1: 101-103, 1979.

Shannon, I. L.: Responses of enamel, dentin and root surfaces to mouthrinse concentrations of sodium fluoride and stannous fluoride. J Dent Child 47: 17-20, 1980.

Snedecor, G. W. and Cochran, W. G.: Statistical Methods. 6th ed. Ames, State Uni Press, Iowa. pp. 271-275, 1976.

Stearns, R. I.: Potential errors in analyzing enamel for fluoride concentrations and rates of acid dissolution subsequent to stannous fluoride treatments. *J Dent Res* 51: 747-755, 1972.

Summerlin, D. J., Retief, D. H. and Denton, J. C.: Enamel fluoride concentration in human mandibular permanent first molars. *J Dent Assoc S Afr* 39: 521-525, 1984.

Svatun, B. and Attramadal, A.: The effect of stannous fluoride on human plaque acidogenicity in situ (Stephan curve). *Acta Odontol Scand* 36: 211-218, 1978.

Tinanoff, N., Brady, J. M. and Gross, A.: The effect of NaF and SnF₂ mouthrinses on bacterial colonization of tooth enamel: TEM and SEM studies. *Caries Res* 10: 415-426, 1976.

Tinanoff, N. and Weeks, D. B.: Current status of SnF₂ as an antiplaque agent. *Pediatr Dent* 1:199-204, 1979.

Tinanoff, N., Klock, B., Camosci, D. A. and Manwell, M. A.: Microbiologic effects of SnF₂ and NaF mouthrinses in subjects with high caries activity. Results after one year. *J Dent Res* 62: 907-911, 1983.

Torell, P. and Siberg, A.: Mouthwash with sodium fluoride and potassium fluoride. *Odont Revy* 13: 62-72, 1962.

Uchtmann, H. and Duschner, H.: Electron spectroscopic studies of interactions between superficially-applied fluorides and surface enamel. *J Dent Res* 61: 423-428, 1982.

van der Merwe, E. H. M., Retief, D. H., Barbakow, F. H. and Friedman, M.: An evaluation of an in vivo enamel acid etch biopsy technique for fluoride determination. *J Dent Assoc S Afr* 29: 81-87, 1974.

Volker, J. F.: The effects of fluoride on the solubility of enamel and dentin. *Proc Soc Exp Biol (NY)* 42: 725, 1939.

Volker, J. F., Hodge, H. C., Wilson, H. J., and van Voochis, S. N.: The adsorption of fluorides by enamel, dentin, bone, and hydroxyapatite as shown by the radioactive isotope. *J Biol Chem* 134: 543-548, 1940.

Volpe, A. R.: Dentifrices and mouth rinses. In A Textbook of Preventive Dentistry, ed. R. E. Stallard, Philadelphia, Saunders Co., pp.170-216, 1982.

Weatherell, J. A. and Hargreaves, J. A.: The micro-sampling of enamel in thin layers by means of strong acids. Arch oral Biol 10: 139-142, 1965.

Weatherell, J. A., Hallsworth, A. S. and Robinson, C.: The effect of tooth wear on the distribution of fluoride in the enamel surface of human teeth. Arch oral Biol 18: 1175-1189, 1973.

Wefel, J. S. and Harless, J. D.: Topical fluoride application and lesion progression in vitro. J Dent Res 11: 1276-1278, 1984.

Wei, S. H. Y. and Forbes, W. C.: Electron microprobe investigations of stannous fluoride reactions with dental enamel. J Dent Res 53: 51-56, 1974.

Wei, S. H. Y.: Scanning electron microscope study of stannous fluoride-treated enamel surfaces. J Dent Res 53: 57-63, 1974.

Wei, S. H. Y.: Effect of topical fluoride solutions on the enamel surface as studied by scanning electron microscopy. Caries Res 9: 445-458, 1975.

Wei, S. H. Y. and Connor, C. W.: Fluoride uptake and retention in vitro following topical fluoride applications. J Dent Res 62: 830-832, 1983.

Weisz, W. S.: The reduction of dental caries through use of a sodium fluoride mouthwash. J Am Dent Assoc 60: 438-456, 1960.

Wolf, R. G.: Effect of a stannous fluoride prophylaxis paste on enamel solubility. J Dent Res 43: 168-174, 1964.

13. TABLES FOR MATERIALS AND METHODS

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Table 1. Details of the test solutions used:

Substance	ppm F ⁻ *	pH*
1. Control (distilled H ₂ O)	-	6.8
2. SnF ₂ (75%) & AmF 297 (25%) & MW**	250	3.6
3. SnF ₂ (50%) & AmF 297 (50%) & MW**	250	4.0
4. SnF ₂ (25%) & AmF 297 (75%) & MW**	250	4.4
5. SnF ₂	250	3.4
6. AmF 297	250	4.0
7. NaF	250	6.0
8. Act [®]	250	7.0
9. Candida [®]	1'500	6.5
10. SnF ₂ (50%) & AmF 297 (50%) & MW**	250	4.0
11. Act [®]	250	6.5
12. Candida [®]	1'500	6.5
13. Control (distilled H ₂ O)	-	6.8

* Determined from 10% solutions.

** MW=Other components of the mouth wash.

Molar Fluoride Ratios (MFR's) of :

Solution 2 was 3 : 1 (SnF₂ : AmF 297)
 Solution 3 was 1 : 1 (SnF₂ : AmF 297)
 Solution 4 was 1 : 3 (SnF₂ : AmF 297)
 Solution 10 was 1 : 1 (SnF₂ : AmF 297)

Table 2. Summary of the Experimental Design

TEST SOLUTIONS	N	AFTER EACH TOPICAL APPLICATION THE SPECIMENS WERE:	
		PER SOLUTION	
Group 1	1 → 9	20	water-washed for 10 sec
Group 2	1 → 9	20	water-washed for 60 sec
Group 3	1 → 9	20	water-washed for 5 min
Group 4	10 → 13	20	water-washed for 10 sec
Group 5	10 → 13	20	water-washed for 10 sec and brushed for 10 sec
Group 6	10 → 13	20	water-washed for 20 sec and brushed for 20 sec

Each enamel specimen was acid-etched 8 times:	
1st = 5 sec	5th = 4 min
2nd = 10 sec	6th = 4 min
3rd = 15 sec	7th = 4 min
4th = 15 sec	8th = 5 min
Total: 17 min 45 sec	

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Table 3 Mean ($\pm$ SD) amount of phosphorus (μ g) dissolved from the first layer etched and cumulatively from the subsequent seven etched layers of the enamel topically treated with the nine test solutions and water-washed for 10 sec after each application (Group 1; Table 2).

Test Solution	N	Cumulative Acid Etches:							
		1st	2nd	3rd	4th	5th	6th	7th	8th
1.	20	17.08 (5.55)	69.91 (16.76)	153.68 (31.18)	241.38 (46.32)	746.04 (178.26)	1242.72 (304.81)	1754.41 (485.44)	2354.17 (695.29)
2.	20	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	4.61 (6.42)	10.49 (14.58)	17.40 (25.59)	27.72 (44.85)
3.	20	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	4.62 (6.87)	10.87 (13.97)	20.54 (22.76)	34.97 (34.67)
4.	20	0.00 (0.00)	0.73 (1.80)	1.91 (4.51)	3.19 (7.59)	31.94 (35.18)	81.50 (71.02)	153.14 (134.11)	307.63 (273.41)
5.	20	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	1.50 (2.75)	3.18 (6.31)	5.25 (11.02)	7.32 (15.13)
6.	20	7.88 (5.52)	38.16 (18.22)	94.69 (42.71)	163.03 (67.64)	603.25 (171.16)	1078.33 (346.76)	1696.41 (388.27)	2372.61 (571.97)
7.	20	16.57 (6.03)	72.32 (16.34)	161.26 (26.85)	251.34 (38.02)	737.05 (125.70)	1213.78 (239.72)	1744.76 (384.74)	2426.76 (641.39)
8.	20	17.95 (5.31)	72.50 (16.81)	156.76 (30.14)	246.77 (41.83)	744.51 (138.19)	1267.74 (257.53)	1819.05 (395.83)	2522.07 (614.81)
9.	20	15.00 (6.53)	63.62 (19.53)	141.15 (35.38)	224.85 (52.12)	671.42 (181.34)	1201.60 (371.09)	1800.10 (545.48)	2569.12 (897.41)

LSD* < 0.05 2.70 8.18 15.71 23.44 75.08 144.18 208.90 327.91
 < 0.01 3.56 10.80 20.73 30.93 99.08 190.27 275.68 432.73
 < 0.001 4.58 13.88 26.45 39.76 127.36 244.58 354.37 556.26

* Least Significant Difference between two means

Table 4 Mean ($\pm$ SD) amount of phosphorus (μ g) dissolved from the first layer etched and cumulatively from the subsequent seven etched layers of the enamel topically treated with the nine test solutions and water-washed for 60 sec after each application (Group 2; Table 2).

Test Solution	N	Cumulative Acid Etches:							
		1st	2nd	3rd	4th	5th	6th	7th	8th
1.	20	17.76 (4.64)	74.16 (15.20)	160.70 (27.97)	250.96 (42.37)	764.43 (228.62)	1288.54 (342.26)	1889.24 (515.45)	2692.75 (820.16)
2.	20	0.00 (0.00)	0.48 (2.08)	1.04 (4.55)	1.86 (6.93)	12.54 (36.87)	26.49 (72.52)	59.15 (178.35)	88.09 (236.87)
3.	20	0.00 (0.00)	0.00 (0.00)	0.62 (2.69)	1.12 (4.32)	8.77 (13.31)	21.83 (27.41)	42.66 (49.41)	77.39 (75.18)
4.	20	0.00 (0.00)	0.72 (1.77)	1.74 (4.34)	2.92 (7.17)	31.82 (19.63)	81.26 (59.70)	179.24 (157.65)	364.25 (263.50)
5.	20	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	1.72 (3.95)	3.17 (7.02)	5.24 (12.28)	7.71 (17.12)
6.	20	10.01 (5.11)	42.14 (14.41)	99.89 (31.08)	171.25 (47.61)	571.80 (155.00)	1084.17 (284.79)	1610.15 (418.32)	2365.64 (577.12)
7.	20	12.80 (6.10)	62.22 (16.69)	145.36 (33.82)	232.77 (50.43)	724.99 (184.49)	1267.72 (326.70)	1898.21 (522.04)	2684.16 (893.98)
8.	20	17.68 (6.25)	74.73 (18.10)	165.30 (32.69)	260.71 (45.21)	787.81 (161.95)	1339.07 (306.65)	1925.38 (523.93)	2677.85 (833.51)
9.	20	15.70 (6.41)	67.89 (18.25)	149.05 (30.67)	232.34 (43.04)	740.49 (177.92)	1314.15 (311.14)	1975.06 (522.51)	2807.90 (837.26)
< 0.05		2.67	7.74	14.64	21.44	84.14	147.93	239.06	379.83
LSD*		< 0.01	3.53	19.32	28.30	112.76	195.22	315.48	501.25
< 0.001		4.53	13.14	24.83	36.37	143.30	250.95	405.53	644.33

* Least Significant Difference between two means

Table 5 Mean ($\pm$ SD) amount of phosphorus (μ g) dissolved from the first layer etched and cumulatively from the subsequent seven etched layers of the enamel topically treated with the nine solutions and water-washed for 5 min after each application (Group 3; Table 2).

Test Solution	N	1st	2nd	Cumulative Acid Etches:					6th	7th	8th
				3rd	4th	5th					
1.	20	14.21 (4.90)	67.43 (13.63)	153.29 (25.76)	242.18 (36.40)	718.98 (147.76)			1206.28 (303.10)	1730.88 (450.98)	2324.03 (620.33)
2.	20	0.00 (0.00)	0.42 (1.83)	1.15 (5.01)	2.01 (8.75)	11.63 (39.22)			24.33 (78.78)	43.99 (130.71)	71.75 (202.11)
3.	20	0.00 (0.00)	0.00 (0.00)	0.86 (2.66)	1.69 (5.22)	7.84 (12.79)			17.65 (27.21)	35.01 (57.35)	62.15 (107.87)
4.	20	0.00 (0.00)	0.15 (0.65)	0.70 (2.11)	2.21 (4.34)	30.30 (18.93)			70.84 (37.05)	132.61 (71.83)	266.32 (165.32)
5.	20	0.00 (0.00)	0.00 (0.00)	0.25 (1.09)	0.48 (2.10)	0.73 (3.19)			1.15 (4.32)	2.27 (6.02)	3.08 (7.72)
6.	20	8.02 (4.31)	40.01 (13.50)	96.88 (29.39)	163.00 (43.42)	572.42 (159.98)			1102.68 (307.89)	1668.19 (460.22)	2353.70 (631.65)
7.	20	10.59 (4.89)	50.06 (10.94)	119.93 (24.96)	194.54 (38.13)	605.12 (134.18)			996.65 (219.25)	1434.36 (316.81)	1990.05 (467.93)
8.	20	16.13 (7.43)	65.68 (15.40)	145.57 (25.67)	230.63 (34.60)	719.25 (86.05)			1163.90 (198.98)	1665.94 (273.06)	2374.54 (512.85)
9.	20	12.15 (3.71)	56.05 (9.76)	125.95 (17.95)	201.71 (25.03)	584.57 (136.66)			943.69 (248.24)	1439.36 (358.31)	2042.08 (462.19)
< 0.05		2.42	5.97	11.71	16.94	63.65			122.09	179.49	259.83
LSD* < 0.01		3.20	7.88	15.46	22.36	84.00			161.12	236.86	342.88
< 0.001		4.11	10.13	19.87	29.48	107.98			207.11	304.47	440.76

* Least Significant Difference between two means.

Table 6 Mean ($\pm$ SD) amount of phosphorus (μ g) dissolved from the first layer etched and cumulatively from the subsequent seven etched layers of the enamel topically treated with the four test solutions and water-washed for 10 sec after each application (Group 4; Table 2)

Test Solution	N	Cumulative Acid Etches:							
		1st	2nd	3rd	4th	5th	6th	7th	8th
10.	20	0.00 (0.00)	0.39 (1.69)	0.96 (4.19)	1.08 (4.70)	11.05 (18.81)	21.49 (31.91)	33.02 (45.39)	58.58 (70.51)
11.	20	16.54 (4.27)	69.06 (14.98)	153.86 (27.50)	240.71 (37.90)	668.68 (166.23)	1105.87 (280.45)	1591.00 (455.98)	2208.73 (734.41)
12.	20	16.12 (4.44)	65.54 (15.44)	140.83 (27.39)	219.07 (39.42)	616.57 (135.52)	1052.78 (241.71)	1489.03 (340.13)	2032.34 (517.30)
13.	20	18.37 (6.62)	72.80 (20.54)	160.33 (32.06)	251.68 (44.02)	731.45 (147.17)	1197.59 (223.27)	1669.37 (366.63)	2307.99 (569.97)
LSD*	< 0.05	2.85	9.38	15.91	22.16	82.13	104.49	213.61	335.76
	< 0.01	3.78	12.45	21.10	29.39	108.95	138.61	283.36	445.41
	< 0.001	4.90	16.13	27.34	38.08	141.16	179.60	367.15	577.12

* Least Significant Difference between two means.

Table 7 Mean ($\pm$ SD) amount of phosphorus (μ g) dissolved from the first layer etched and cumulatively from the subsequent seven etched layers of the enamel topically treated with the four test solutions then water-washed for 10 sec and brushed for 10 sec after each application (Group 5; Table 2).

Test Solution	N	Cumulative Acid Etches:							
		1st	2nd	3rd	4th	5th	6th	7th	8th
10.	20	11.47 (6.85)	54.29 (19.35)	128.59 (38.76)	211.78 (57.96)	683.16 (186.24)	1161.01 (347.51)	1708.91 (528.86)	2403.91 (854.44)
11.	20	16.12 (6.85)	66.70 (17.30)	147.49 (31.00)	235.32 (44.03)	760.05 (129.86)	1303.87 (255.01)	1914.58 (427.95)	2682.97 (774.93)
12.	20	16.12 (7.77)	62.07 (20.33)	134.76 (34.18)	210.49 (49.95)	672.60 (130.33)	1233.65 (232.44)	1822.12 (407.13)	2625.35 (681.91)
13.	20	21.86 (5.67)	80.79 (13.78)	170.65 (25.02)	262.55 (35.20)	767.83 (127.74)	1348.71 (206.92)	1979.25 (378.83)	2907.54 (704.31)
LSD*	<0.05 <0.01 <0.001	4.30 5.70 7.39	11.25 14.93 19.34	20.55 27.26 35.32	29.93 39.70 51.44	91.73 121.69 157.68	167.42 222.09 287.77	276.71 367.07 475.62	476.73 632.40 819.41

* Least Significant Difference between two means.

Table 8

Mean ($\pm$ SD) amount of phosphorus (μ g) dissolved from the first layer etched and cumulatively from the subsequent seven etched layers of the enamel topically treated with the four solutions and water-washed for 20 sec and brushed for 20 sec after each application (Group 6; Table 2)

Test

Cumulative Acid Etches:

Solution	N	1st	2nd	3rd	4th	5th	6th	7th	8th
10.	20	9.29 (5.64)	45.64 (14.90)	110.29 (28.98)	184.16 (43.32)	665.55 (124.81)	1182.76 (206.91)	1759.95 (375.70)	2560.94 (601.18)
11.	20	14.09 (5.08)	60.93 (11.59)	134.36 (21.30)	210.85 (29.71)	657.68 (107.20)	1083.25 (202.79)	1598.08 (351.13)	2278.88 (548.10)
12.	20	12.86 (5.61)	54.16 (16.12)	120.69 (29.99)	190.01 (44.20)	585.28 (146.42)	1044.73 (239.17)	1518.76 (296.53)	2178.45 (440.08)
13.	20	20.21 (4.39)	74.48 (15.12)	157.29 (32.83)	247.42 (48.30)	816.89 (335.47)	1353.01 (438.27)	1884.18 (583.75)	2670.20 (810.59)
LSD*	<0.05 <0.01 <0.001	3.28 4.35 5.64	9.15 12.14 15.73	18.01 23.89 30.96	26.40 35.02 45.37	126.38 167.65 217.22	181.78 241.15 312.45	262.18 347.80 450.65	387.30 513.78 665.71

* Least Significant Difference between two means.

Table 9 Mean ($\pm$ SD) enamel fluoride concentration (ppm) in the first layer etched and cumulatively in the subsequent seven etched layers of the enamel topically treated with the nine test solutions and water-washed for 10 sec after each application (Group 1, Table 2).

Test Solution	N	1st	2nd	3rd	Cumulative Acid Etches:				7th	8th
					4th	5th	6th			
1.	20	618 (180)	350 (92)	228 (56)	172 (42)	39 (18)	55 (13)	45 (11)	39 (10)	
2.	20	0 (0)	0 (0)	0 (0)	0 (0)	404 (275)	214 (246)	402 (388)	404 (319)	
3.	20	0 (0)	0 (0)	0 (0)	0 (0)	440 (476)	427 (406)	427 (335)	440 (241)	
4.	20	0 (0)	188 (500)	236 (504)	230 (481)	322 (254)	467 (181)	392 (148)	322 (132)	
5.	20	0 (0)	0 (0)	0 (0)	0 (0)	46 (198)	69 (193)	62 (173)	46 (127)	
6.	20	1875 (1154)	1683 (580)	1509 (313)	1321 (212)	255 (151)	452 (84)	323 (60)	255 (42)	
7.	20	1032 (243)	626 (108)	430 (84)	335 (77)	69 (40)	104 (25)	82 (20)	69 (17)	
8.	20	1106 (321)	670 (146)	449 (108)	345 (84)	68 (40)	104 (28)	81 (22)	68 (17)	
9.	20	1551 (331)	1023 (176)	774 (110)	643 (81)	134 (58)	224 (54)	165 (41)	134 (34)	
LSD*	< 0.05	359	180	102	72	137	115	118	92	
	< 0.01	475	238	135	96	181	152	156	96	
	< 0.001	614	307	175	124	233	196	200	156	

* Least Significant Difference between two means

Table 10

Mean ($\pm$ SD) enamel fluoride concentration (ppm) in the first layer etched and cumulatively in the subsequent seven etched layers of the enamel topically treated with the nine test solutions and water-washed for 60 sec after each application (Group 2; Table 2).

Test Solution	N	Cumulative Acid Etches:							
		1st	2nd	3rd	4th	5th	6th	7th	8th
1.	20	673 (232)	384 (156)	253 (113)	193 (91)	90 (46)	61 (32)	48 (24)	40 (20)
2.	20	0 (0)	29 (125)	24 (105)	102 (281)	293 (415)	294 (334)	292 (332)	360 (350)
3.	20	0 (0)	0 (0)	21 (91)	60 (187)	461 (414)	376 (173)	482 (321)	425 (285)
4.	20	0 (0)	128 (306)	32 (140)	444 (1641)	432 (119)	362 (103)	303 (101)	231 (76)
5.	20	0 (0)	0 (0)	0 (0)	0 (0)	78 (285)	52 (188)	38 (143)	43 (133)
6.	20	1954 (842)	1791 (499)	1687 (733)	1442 (423)	821 (199)	570 (214)	423 (197)	315 (141)
7.	20	1185 (992)	672 (105)	453 (70)	345 (68)	149 (44)	101 (33)	77 (22)	65 (19)
8.	20	902 (258)	496 (120)	322 (92)	240 (76)	103 (36)	70 (26)	56 (20)	46 (17)
9.	20	1447 (312)	973 (132)	751 (99)	639 (98)	321 (81)	209 (57)	157 (47)	126 (33)
LSD*	< 0.05	388	158	213	128	146	102	111	104
	< 0.01	514	209	282	169	192	134	147	137
	< 0.001	664	270	364	219	247	172	189	176

* Least Significant Difference between two means

Table 11 Mean ($\pm$ SD) enamel fluoride concentration (ppm) in the first layer etched and cumulatively in the subsequent seven etched layers of the enamel topically treated with the nine test solutions and water-washed for 5 min after each application (Group 3; Table 2).

Test Solution	N	Cumulative Acid Etches:							
		1st	2nd	3rd	4th	5th	6th	7th	8th
1.	20	721 (227)	389 (112)	255 (75)	191 (58)	41 (26)	61 (22)	48 (16)	41 (14)
2.	20	0 (0)	44 (192)	33 (144)	26 (114)	260 (240)	180 (255)	279 (279)	260 (213)
3.	20	0 (0)	0 (0)	56 (184)	51 (159)	268 (317)	248 (201)	259 (177)	268 (128)
4.	20	0 (0)	84 (367)	120 (369)	197 (370)	240 (171)	341 (135)	293 (115)	240 (107)
5.	20	0 (0)	0 (0)	79 (343)	66 (288)	100 (272)	112 (301)	106 (276)	100 (262)
6.	20	1493 (783)	1439 (275)	1366 (335)	1174 (257)	211 (114)	384 (84)	275 (61)	211 (50)
7.	20	1216 (444)	749 (231)	502 (158)	379 (127)	75 (73)	115 (47)	91 (38)	75 (30)
8.	20	843 (299)	487 (120)	315 (85)	233 (72)	41 (41)	65 (27)	52 (23)	41 (18)
9.	20	1310 (288)	888 (170)	695 (130)	590 (124)	145 (132)	259 (118)	194 (86)	150 (62)
LSD*	< 0.05	286	121	115	92	114	102	96	81
	< 0.01	378	160	152	121	150	134	126	106
	< 0.001	488	206	243	157	193	172	162	137

* Least Significant Difference between two means

Table 12 Mean ($\pm$ SD) enamel fluoride concentration (ppm) in the first layer etched and cumulatively in the subsequent seven etched layers of the enamel topically treated with the four test solutions and water-washed for 10 sec after each application (Group 4; Table 2).

Test Solution	N	Cumulative Acid Etches:							
		1st	2nd	3rd	4th	5th	6th	7th	8th
10.	20	0 (0)	0 (0)	24 (106)	21 (93)	469 (583)	454 (452)	393 (414)	469 (596)
11.	20	1115 (485)	679 (297)	450 (192)	344 (160)	81 (79)	116 (63)	94 (55)	81 (50)
12.	20	1415 (339)	996 (181)	784 (133)	672 (117)	193 (104)	299 (74)	232 (65)	193 (56)
13.	20	805 (479)	518 (339)	328 (179)	248 (132)	55 (52)	80 (35)	65 (29)	55 (25)
LSD*	< 0.05 < 0.01 < 0.001	240 317 412	153 203 263	99 131 169	81 107 135	189 251 325	146 194 251	133 177 229	189 251 326

* Least Significant Difference between two means

Table 13 Mean ($\pm$ SD) enamel fluoride concentration (ppm) in the first layer etched and cumulatively in the subsequent seven etched layers of the enamel topically treated with the four test solutions water-washed for 10 sec and brushed for 10 sec after each application (Group 5; Table 2).

Test Solution		Cumulative Acid Etches:							
	N	1st	2nd	3rd	4th	5th	6th	7th	8th
10.	20	920 (889)	818 (181)	643 (126)	503 (126)	205 (64)	136 (46)	101 (32)	82 (22)
11.	20	927 (340)	698 (122)	522 (86)	408 (78)	166 (43)	109 (28)	83 (20)	69 (15)
12.	20	1137 (309)	1032 (186)	931 (135)	875 (121)	577 (88)	367 (61)	274 (56)	214 (43)
13.	20	330 (204)	241 (158)	167 (121)	127 (93)	57 (39)	38 (25)	30 (20)	26 (17)
LSD*	< 0.05	321	103	75	67	39	27	22	17
	< 0.01	427	137	99	89	52	36	29	22
	< 0.001	553	177	128	115	67	46	38	29

* Least Significant Difference between two means

Table 14

Mean ($\pm$ SD) enamel fluoride concentration (ppm) in the first layer etched and cumulatively in the subsequent seven etched layers of the enamel topically treated with the four test solutions and water-washed for 20 sec and brushed for 20 sec after each application (Group 6; Table 2).

Test Solution	N	Cumulative Acid Etches:							
		1st	2nd	3rd	4th	5th	6th	7th	8th
10.	20	893 (343)	829 (204)	690 (160)	566 (158)	83 (87)	140 (49)	105 (36)	83 (30)
11.	20	899 (197)	703 (154)	583 (112)	508 (102)	104 (62)	169 (42)	130 (35)	104 (28)
12.	20	1073 (390)	978 (196)	874 (155)	815 (139)	252 (143)	427 (114)	324 (89)	252 (67)
13.	20	453 (204)	368 (143)	294 (128)	235 (112)	48 (55)	70 (36)	58 (30)	48 (23)
LSD*	< 0.05	186	111	88	82	59	43	33	26
	< 0.01	247	147	117	108	78	57	44	34
	< 0.001	320	191	152	140	101	73	58	44

* Least Significant Difference between two means

Table 15 Mean ($\pm$ SD) depth of etch (μ m) of the enamel in the first layer etched and cumulatively in the subsequent seven etched layers after being topically treated with the nine test solutions and water-washed for 10 sec after each application (Group 1; Table 2).

Test Solution	N	Cumulative Acid Etches:							
		1st	2nd	3rd	4th	5th	6th	7th	8th
1.	20	4.35 (1.41)	17.80 (4.27)	39.13 (7.94)	61.46 (11.79)	189.97 (45.39)	316.44 (77.62)	446.74 (123.61)	595.17 (181.89)
2.	20	0 (0)	0 (0)	0 (0)	0 (0)	1.17 (1.64)	2.59 (3.75)	4.41 (6.52)	7.87 (11.67)
3.	20	0 (0)	0 (0)	0 (0)	0 (0)	1.18 (1.75)	2.77 (3.56)	5.23 (5.80)	9.69 (9.24)
4.	20	0 (0)	0.19 (0.46)	0.49 (1.15)	0.81 (1.93)	8.62 (8.69)	20.75 (18.09)	39.00 (34.15)	78.33 (69.62)
5.	20	0 (0)	0 (0)	0 (0)	0 (0)	0.38 (0.70)	0.81 (1.61)	1.34 (2.81)	1.86 (3.85)
6.	20	2.01 (1.41)	9.45 (4.77)	24.13 (10.89)	41.51 (17.22)	153.61 (43.58)	287.58 (68.00)	431.97 (98.87)	604.15 (145.64)
7.	20	4.22 (1.53)	18.41 (4.16)	41.06 (6.84)	64.00 (9.68)	187.68 (32.01)	309.07 (61.04)	444.29 (97.98)	617.94 (163.32)
8.	20	4.57 (1.35)	18.46 (4.28)	39.92 (7.68)	62.84 (10.65)	189.58 (35.19)	322.83 (65.57)	463.19 (100.79)	642.21 (156.55)
9.	20	3.82 (1.66)	16.20 (4.97)	35.94 (9.01)	57.25 (13.27)	170.99 (46.18)	305.97 (94.49)	458.37 (138.90)	654.19 (228.51)
LSD*	< 0.05	0.928	2.825	5.390	8.035	19.112	34.792	53.194	83.952
	< 0.01	1.229	3.741	7.136	10.543	25.221	45.913	70.198	110.787
	< 0.001	1.588	4.833	9.220	13.747	32.420	59.019	90.236	142.411

* Least Significant Difference between two means

Table 16 Mean ($\pm$ SD) depth of etch (μ m) of the enamel in the first layer etched and cumulatively in the subsequent seven etched layers after being topically treated with the nine test solutions and water-washed for 60 sec after each application (Group 2; Table 2).

Test Solution		Cumulative Acid Etches:								
	Solution	N	1st	2nd	3rd	4th	5th	6th	7th	8th
1.		20	4.52 (1.18)	18.88 (3.87)	40.97 (7.11)	63.91 (10.79)	194.97 (58.21)	328.11 (87.15)	481.23 (131.35)	685.67 (208.84)
2.		20	0 (0)	0.12 (0.53)	0.27 (1.16)	0.47 (1.77)	3.19 (9.39)	6.75 (18.47)	15.06 (45.41)	22.43 (60.32)
3.		20	0 (0)	0 (0)	0.16 (0.68)	0.29 (1.10)	2.15 (3.42)	5.56 (6.98)	10.86 (12.58)	19.71 (19.15)
4.		20	0 (0)	0.18 (0.45)	0.44 (1.10)	0.74 (1.83)	8.10 (5.00)	21.39 (15.00)	45.64 (40.14)	92.75 (67.10)
5.		20	0 (0)	0 (0)	0 (0)	0 (0)	0.44 (1.01)	0.81 (1.79)	1.33 (3.13)	1.96 (4.36)
6.		20	2.55 (1.30)	10.73 (3.67)	25.42 (7.93)	43.61 (12.12)	145.60 (39.47)	266.90 (72.52)	410.03 (106.52)	602.38 (146.96)
7.		20	3.26 (1.55)	15.84 (4.25)	37.02 (8.61)	59.27 (12.84)	184.61 (46.98)	322.31 (82.48)	483.35 (132.93)	683.49 (227.64)
8.		20	4.50 (1.59)	19.03 (4.61)	42.09 (8.32)	66.39 (11.51)	200.61 (41.24)	340.98 (78.08)	489.82 (133.25)	681.88 (212.24)
9.		20	4.00 (1.66)	17.28 (4.64)	37.96 (7.81)	59.16 (10.96)	188.56 (45.30)	334.63 (79.23)	502.92 (133.05)	714.99 (213.57)
LSD*		<0.05 <0.01 <0.001	0.919 1.216 1.571	2.653 3.513 4.539	5.006 6.629 8.565	7.326 9.700 12.533	21.855 28.841 37.073	37.598 49.617 63.780	60.867 80.324 103.252	96.718 127.635 164.068

* Least Significant Difference between two means

Table 17 Mean ($\pm$ SD) depth of etch (μ m) of the enamel in the first layer etched and cumulatively in the subsequent seven etched layers after being topically treated with the nine test solutions and water-washed for 5 min after each application (Group 3; Table 2).

Test Solution	N	Cumulative Acid Etches:							
		1st	2nd	3rd	4th	5th	6th	7th	8th
1.	20	3.62 (1.25)	17.18 (3.48)	39.03 (6.56)	61.67 (9.27)	173.08 (50.54)	307.17 (77.18)	440.75 (114.84)	591.78 (157.96)
2.	20	0 (0)	0 (0)	0.29 (1.28)	0.51 (2.23)	2.96 (9.99)	6.20 (20.06)	11.20 (33.28)	18.27 (51.47)
3.	20	0 (0)	0 (0)	0.22 (0.68)	0.43 (1.33)	2.00 (3.72)	4.50 (8.83)	8.91 (34.57)	15.82 (27.82)
4.	20	0 (0)	0 (0)	0.54 (0.17)	1.11 (1.11)	4.82 (4.82)	9.43 (9.43)	18.29 (18.29)	42.10 (42.10)
5.	20	0 (0)	0 (0)	0.06 (0.28)	0.12 (0.53)	0.19 (0.81)	0.39 (1.10)	0.58 (1.53)	0.78 (1.97)
6.	20	2.04 (1.10)	10.22 (3.43)	24.67 (7.48)	41.51 (11.06)	145.76 (40.74)	279.88 (78.68)	424.78 (117.19)	599.34 (160.84)
7.	20	2.70 (1.25)	12.75 (2.79)	30.54 (6.36)	49.54 (9.71)	154.09 (34.17)	258.55 (49.98)	365.24 (80.67)	506.74 (119.15)
8.	20	4.10 (1.89)	16.73 (3.92)	37.07 (6.53)	58.73 (8.81)	183.15 (21.91)	302.12 (38.11)	424.20 (69.54)	603.14 (132.20)
9.	20	3.09 (0.94)	14.27 (2.49)	32.07 (4.57)	51.36 (6.44)	148.85 (34.80)	247.94 (63.21)	365.88 (91.29)	519.99 (17.69)
LSD*	<0.05 <0.01 <0.001	0.832 1.102 1.424	2.048 2.712 3.504	4.002 5.296 6.843	5.765 7.633 9.862	17.664 23.311 29.965	29.890 39.444 50.704	45.708 60.319 77.538	66.300 87.493 112.468

* Least Significant Difference between two means

Table 18 Mean ($\pm$ SD) depth of etch (μ m) of the enamel in the first layer etched and cumulatively in the subsequent seven etched layers after being topically treated with the four test solutions and water-washed for 10 sec after each application (Group 4; Table 2).

Test Solution		Cumulative Acid Etches:								
		N	1st	2nd	3rd	4th	5th	6th	7th	8th
10.		20	0 (0)	0.10 (0.43)	0.25 (1.07)	0.39 (1.71)	2.82 (4.79)	5.47 (8.13)	8.41 (11.56)	14.92 (17.96)
11.		20	4.22 (1.10)	17.58 (3.81)	39.18 (7.00)	61.29 (9.65)	170.27 (42.33)	281.59 (71.41)	405.13 (116.11)	562.42 (187.01)
12.		20	4.11 (1.13)	16.69 (3.93)	31.98 (6.96)	55.78 (10.04)	156.99 (34.51)	268.13 (61.56)	379.16 (86.61)	517.51 (131.72)
13.		20	4.68 (1.67)	18.53 (5.23)	40.33 (8.80)	64.09 (11.21)	186.25 (37.48)	304.95 (56.85)	425.08 (93.36)	587.54 (145.14)
LSD*		<0.05	0.853	2.388	4.180	5.655	7.641	34.860	54.391	85.49
		<0.01	1.131	3.168	5.545	7.502	17.256	46.118	72.153	113.418
		<0.001	1.466	4.105	7.184	9.720	22.359	59.756	93.490	146.957

* Least Significant Difference between two means

Table 19 Mean ($\pm$ SD) depth of etch (μ m) of the first layer etched and cumulatively in the subsequent seven etched layers after being topically treated with the four test solutions and water-washed for 10 sec and brushed for 10 sec after each application (Group 5; Table 2).

Test Solution	N	Cumulative Acid Etches:							
		1st	2nd	3rd	4th	5th	6th	7th	8th
10.	20	2.92 (1.75)	13.84 (4.93)	32.77 (9.85)	53.91 (14.76)	172.52 (47.86)	295.64 (88.49)	435.17 (134.67)	611.96 (217.57)
11.	20	4.10 (1.74)	16.53 (5.53)	37.53 (7.89)	59.94 (11.21)	193.53 (33.07)	333.70 (64.94)	487.52 (108.97)	683.17 (197.33)
12.	20	4.08 (1.98)	15.79 (5.18)	34.31 (8.70)	53.58 (12.72)	171.29 (33.19)	309.80 (59.28)	463.99 (103.67)	668.52 (173.64)
13.	20	5.70 (1.47)	20.55 (3.51)	43.43 (6.37)	66.76 (8.88)	195.53 (32.53)	343.41 (52.69)	504.00 (96.46)	740.36 (179.34)

<0.05	1.098	3.052	5.229	7.611	23.447	42.645	70.46	121.391
LSD*	1.456	4.049	6.937	10.097	31.104	56.571	93.470	161.032
<0.001	1.887	5.246	8.988	13.082	40.301	73.299	121.111	208.651

* Least Significant Difference between two means.

Table 20 Mean ($\pm$ SD) depth of etch (μ m) of the enamel in the first layer etched and cumulatively in the subsequent seven etched layers after being topically treated with the four test solutions and water-washed for 20 sec and brushed for 20 sec after each application (Group 6; Table 2).

Test Solution	N	Cumulative Acid Etches:							
		1st	2nd	3rd	4th	5th	6th	7th	8th
10.	20	2.37 (1.44)	11.62 (3.79)	28.08 (7.38)	46.90 (11.03)	167.14 (32.89)	301.17 (52.69)	448.14 (95.67)	652.74 (151.42)
11.	20	3.59 (1.30)	15.53 (2.94)	34.21 (5.43)	53.69 (7.49)	167.67 (27.36)	275.84 (51.64)	406.93 (89.41)	580.29 (139.57)
12.	20	3.28 (1.43)	13.79 (4.11)	30.73 (7.64)	48.38 (11.26)	149.03 (37.28)	266.03 (60.90)	386.73 (75.51)	554.71 (112.06)
13.	20	5.15 (1.12)	18.97 (3.85)	40.04 (8.38)	63.00 (12.30)	208.01 (85.43)	344.53 (111.60)	479.78 (148.65)	679.93 (206.41)
LSD*	<0.05	0.835	2.330	4.590	6.722	32.297	46.288	66.761	98.366
	<0.01	1.108	3.091	6.088	8.918	42.843	61.404	88.562	130.488
	<0.001	1.436	4.005	7.889	11.555	55.512	79.562	114.751	169.074

* Least Significant Difference between two means

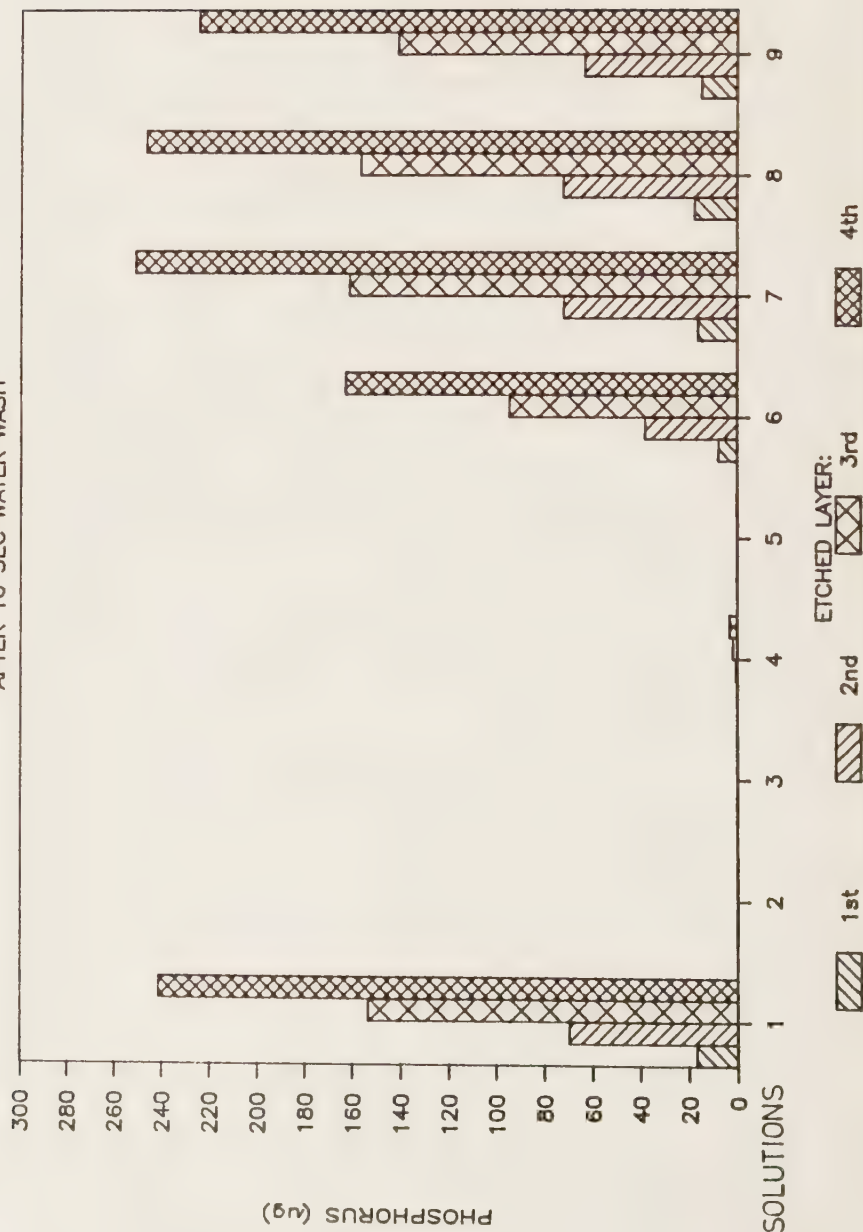
15. BAR DIAGRAMS

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15.2. Figs. 13 - 24: Fluoride Concentrations	95
15.3. Figs. 25 - 36: Depths of Etch	107

FIG. 1

ENAMEL DISSOLUTION RATE: CUMULATIVE

AFTER 10 SEC WATER WASH



ENAMEL DISSOLUTION RATE: CUMULATIVE

AFTER 10 SEC WATER WASH

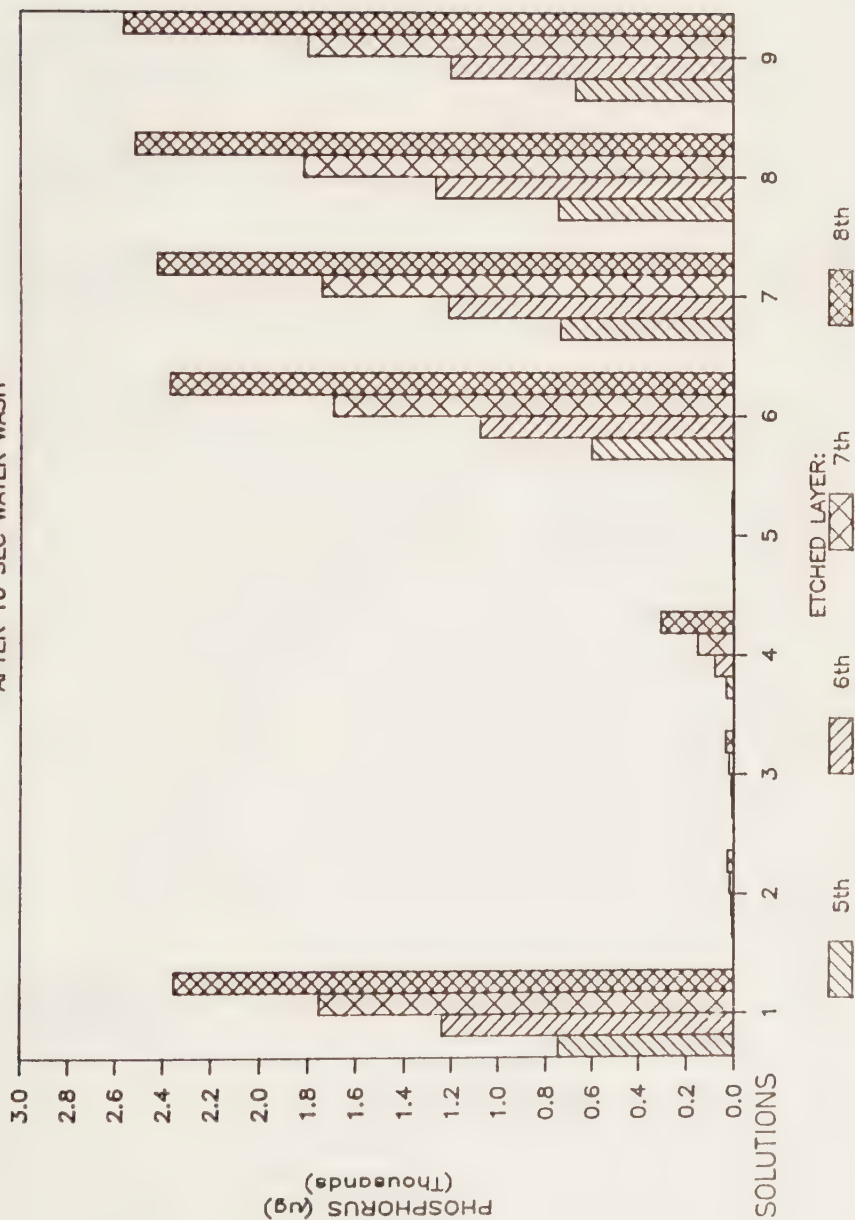
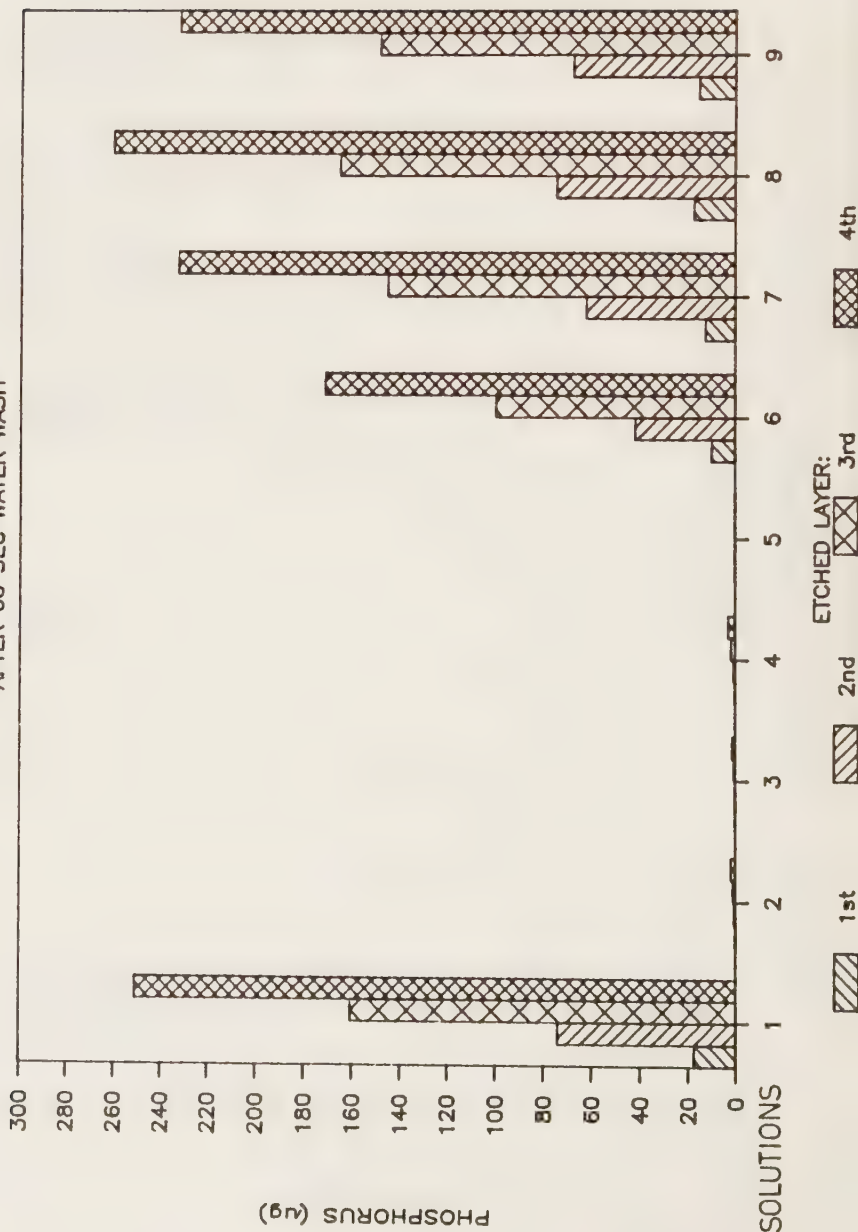


FIG. 2

FIG. 3

ENAMEL DISSOLUTION RATE: CUMULATIVE

AFTER 60 SEC WATER WASH



ENAMEL DISSOLUTION RATE: CUMULATIVE

AFTER 60 SEC WATER WASH

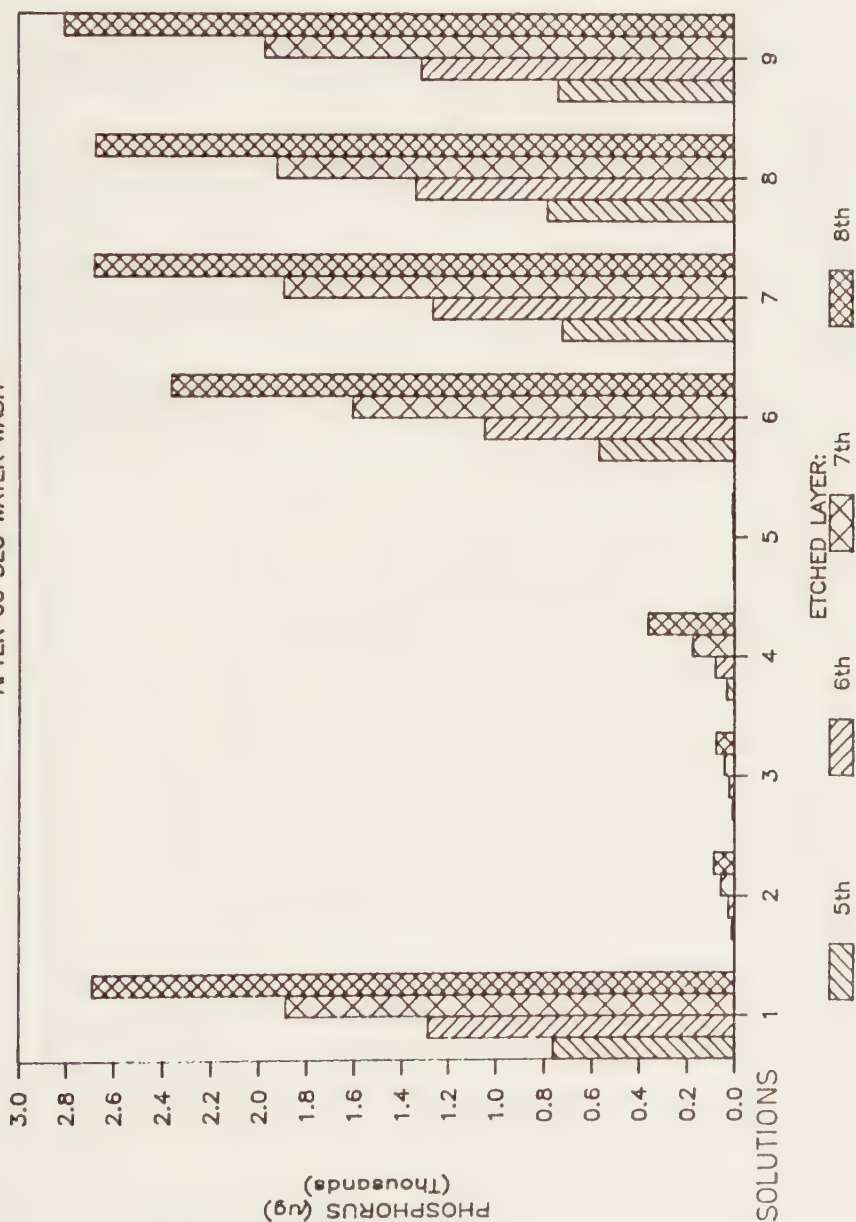


FIG. 4

FIG. 5

ENAMEL DISSOLUTION RATE: CUMULATIVE

AFTER 5 MIN WATER WASH

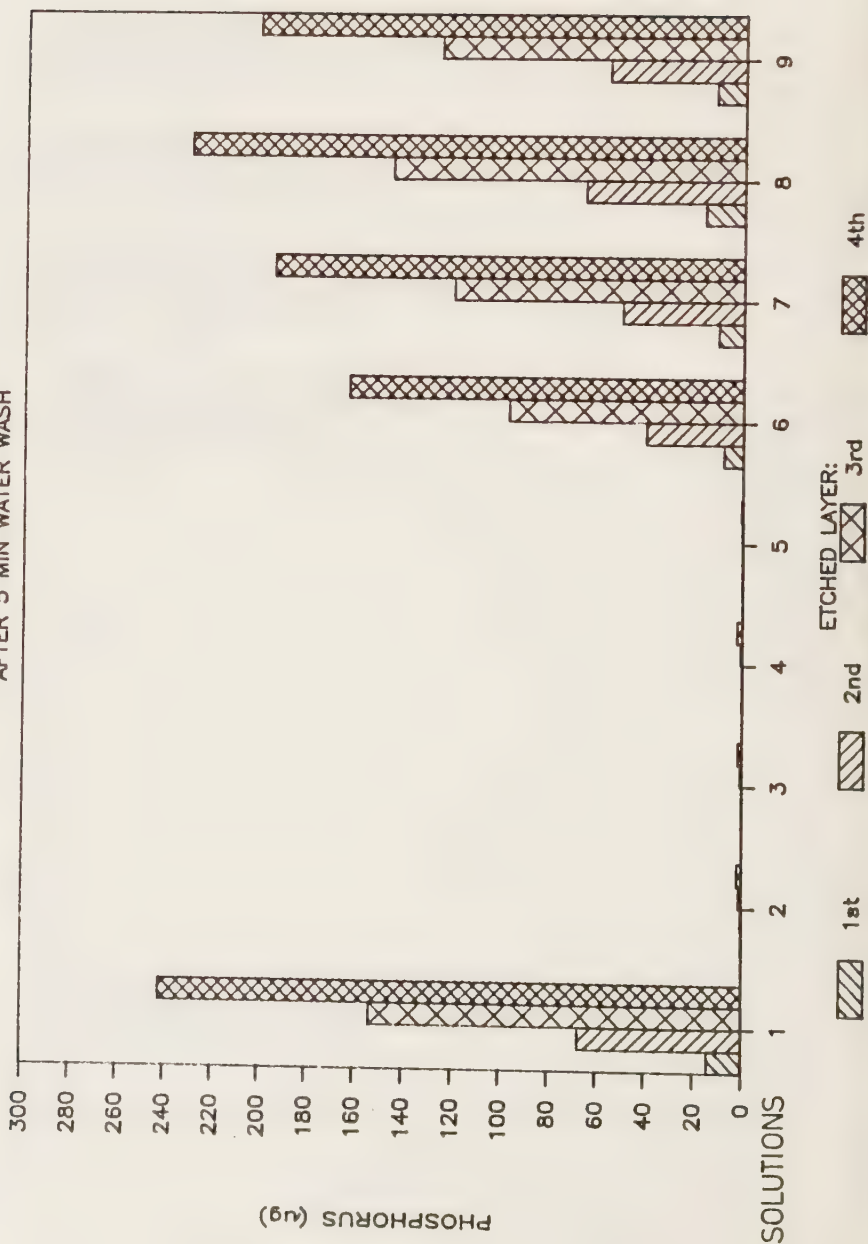


FIG. 6

ENAMEL DISSOLUTION RATE: CUMULATIVE

AFTER 5 MIN WATER WASH

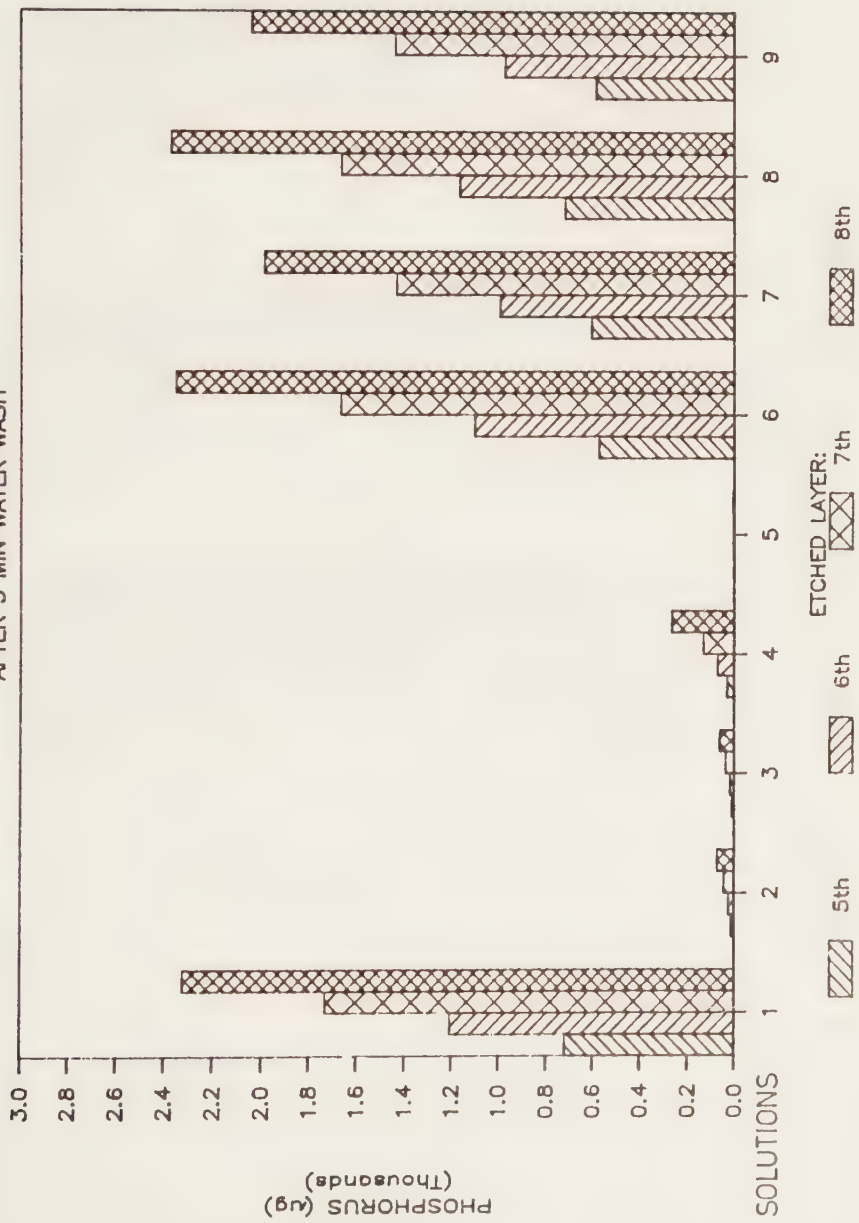


FIG. 7

ENAMEL DISSOLUTION RATE: CUMULATIVE

AFTER 10 SEC WATER WASH

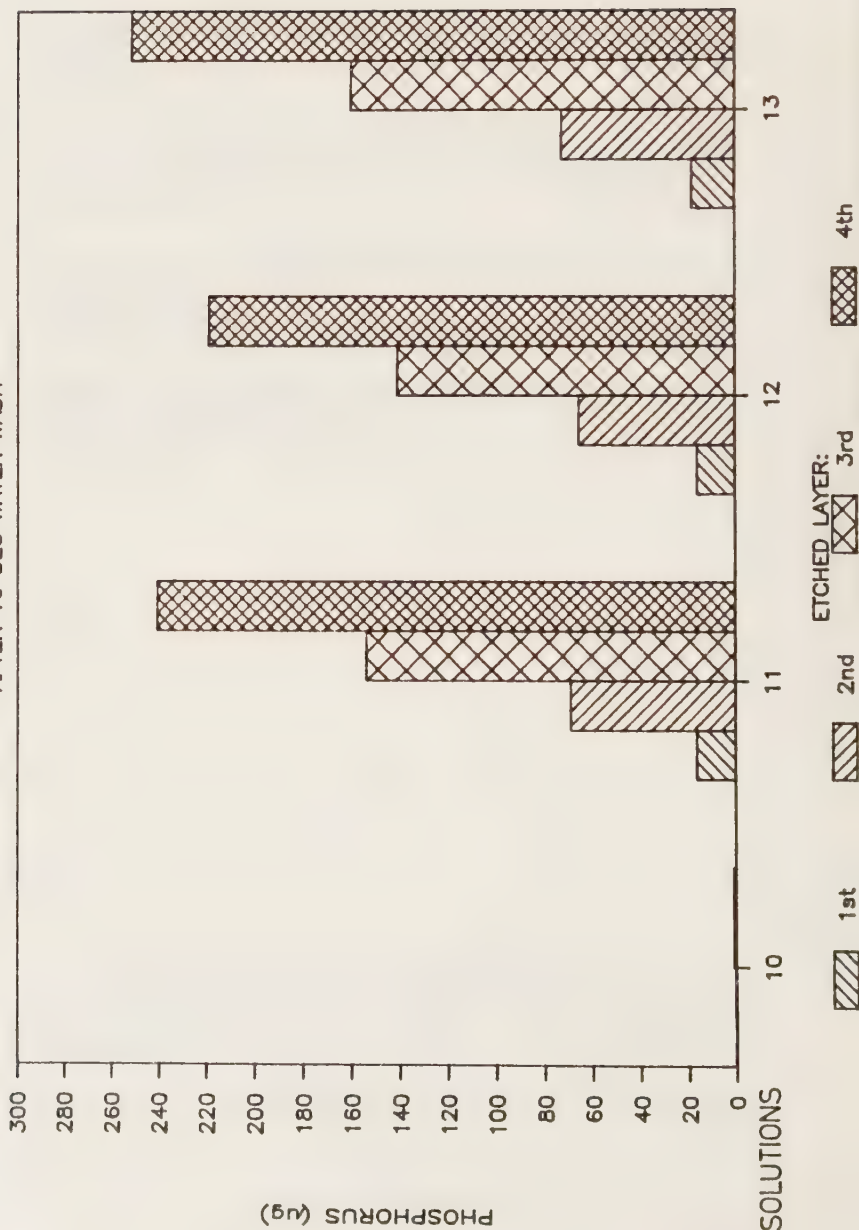


FIG. 8

ENAMEL DISSOLUTION RATE: CUMULATIVE

AFTER 10 SEC WATER WASH

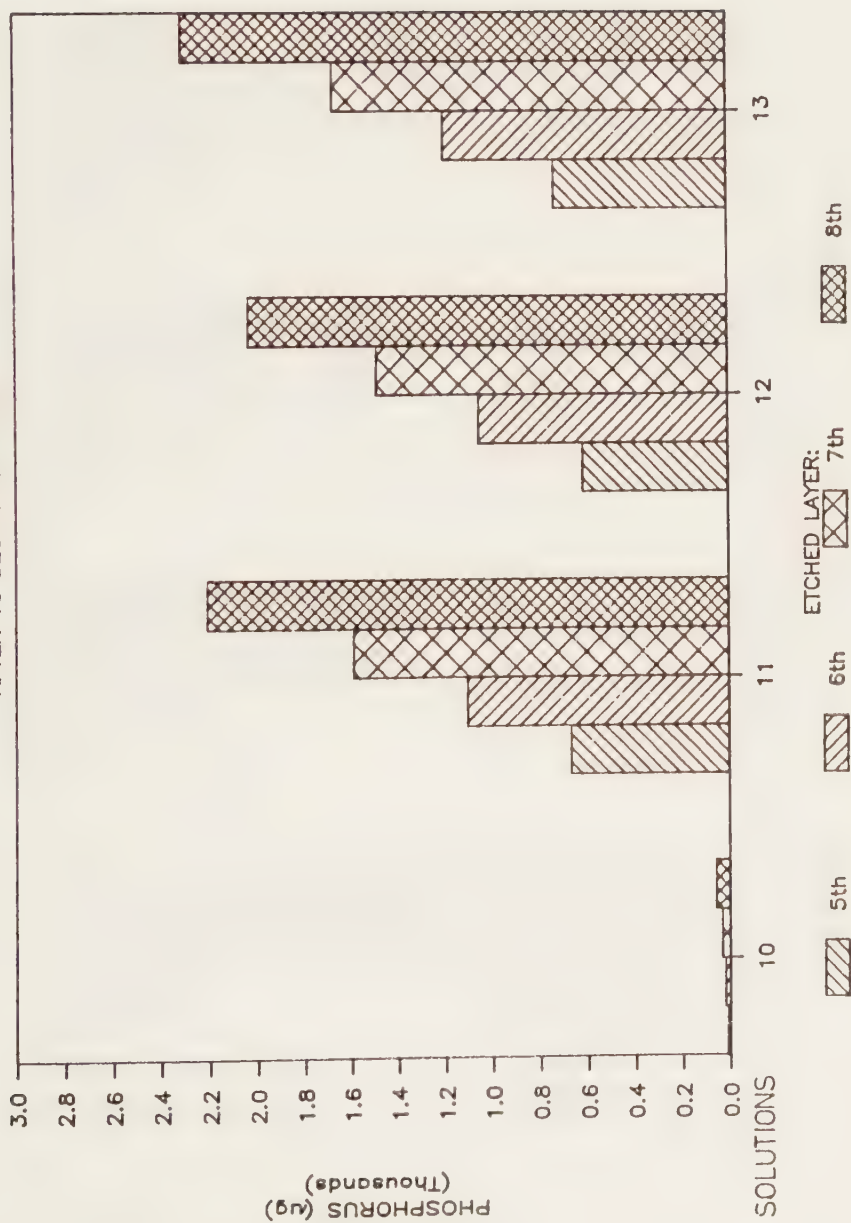


FIG. 9

ENAMEL DISSOLUTION RATE: CUMULATIVE AFTER 10 SEC WATER WASH + 10 SEC BRUSH

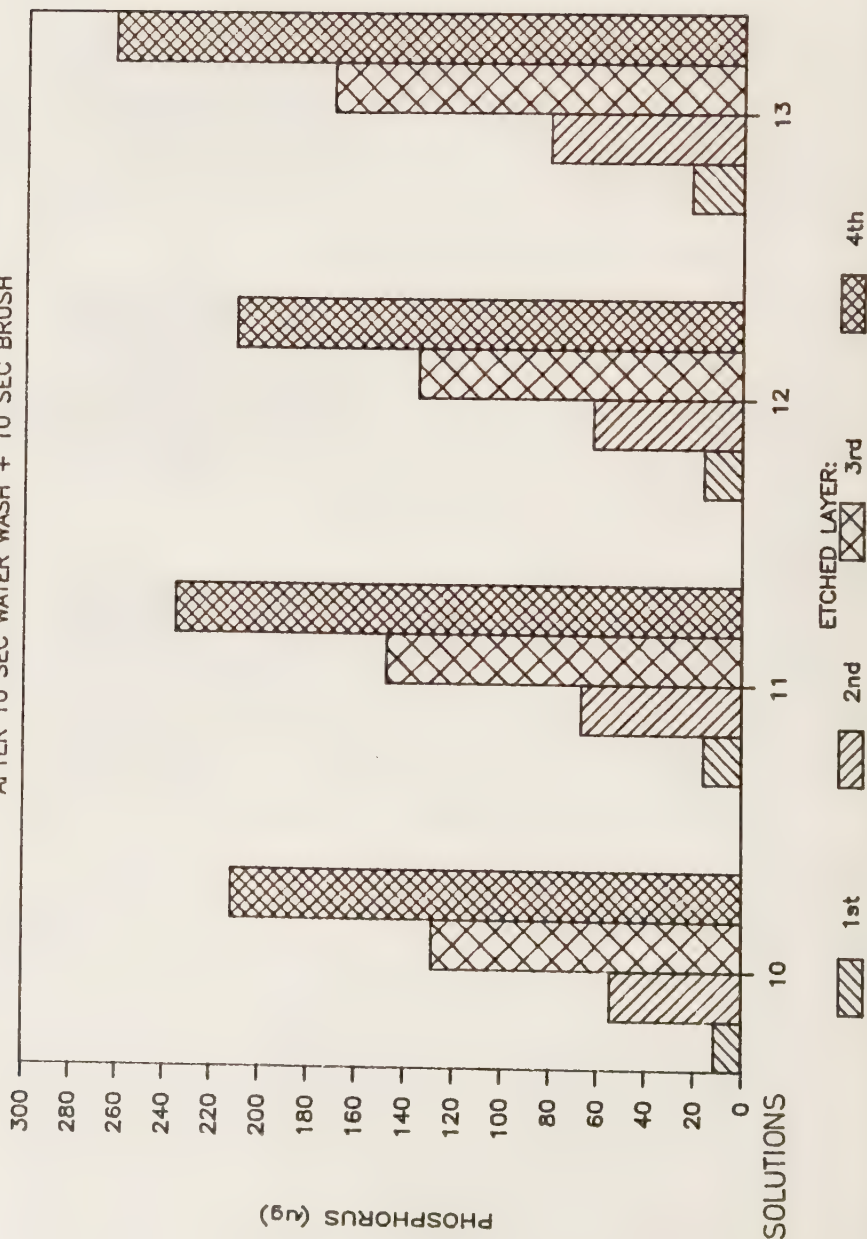


FIG. 10

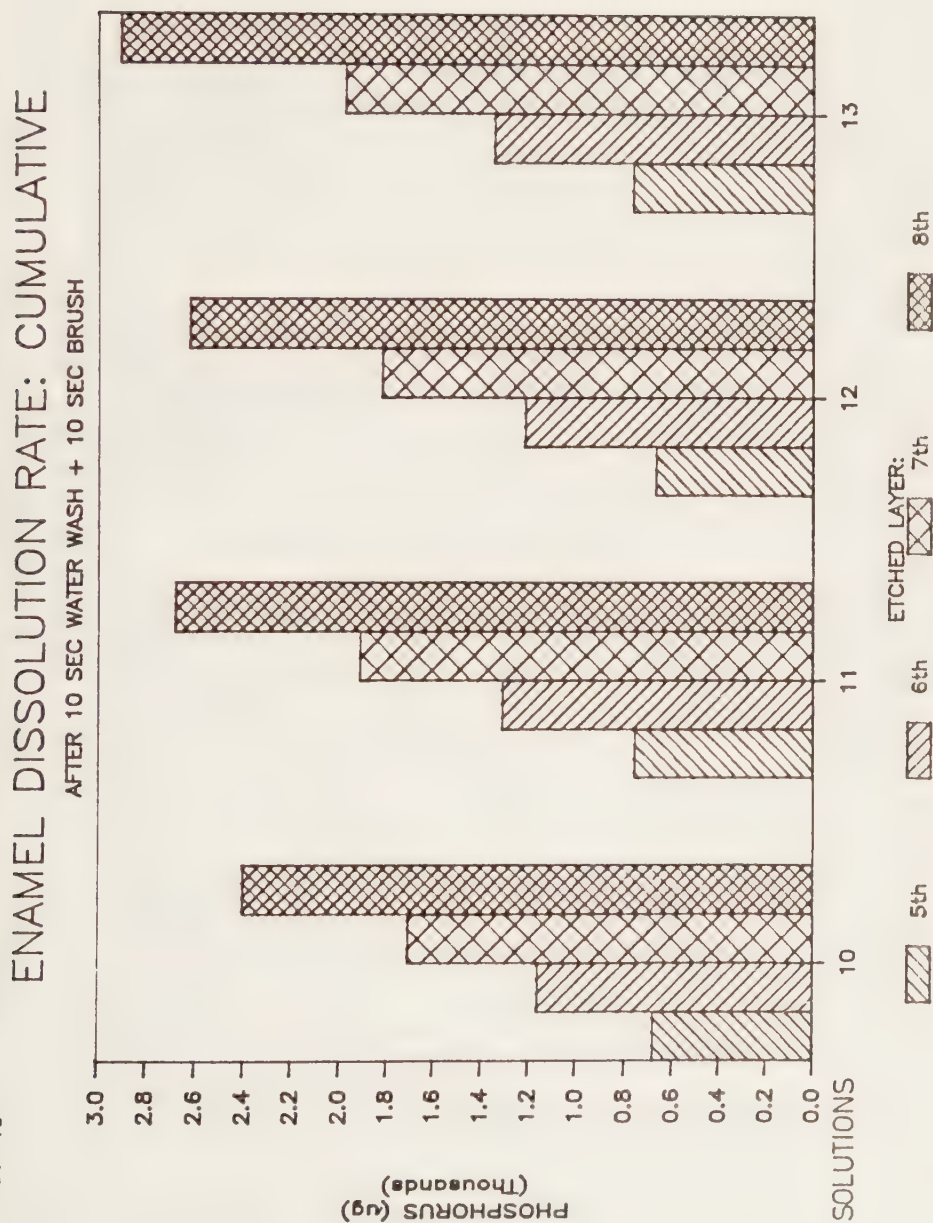


FIG. 11

ENAMEL DISSOLUTION RATE: CUMULATIVE

AFTER 20 SEC WATER WASH + 20 SEC BRUSH

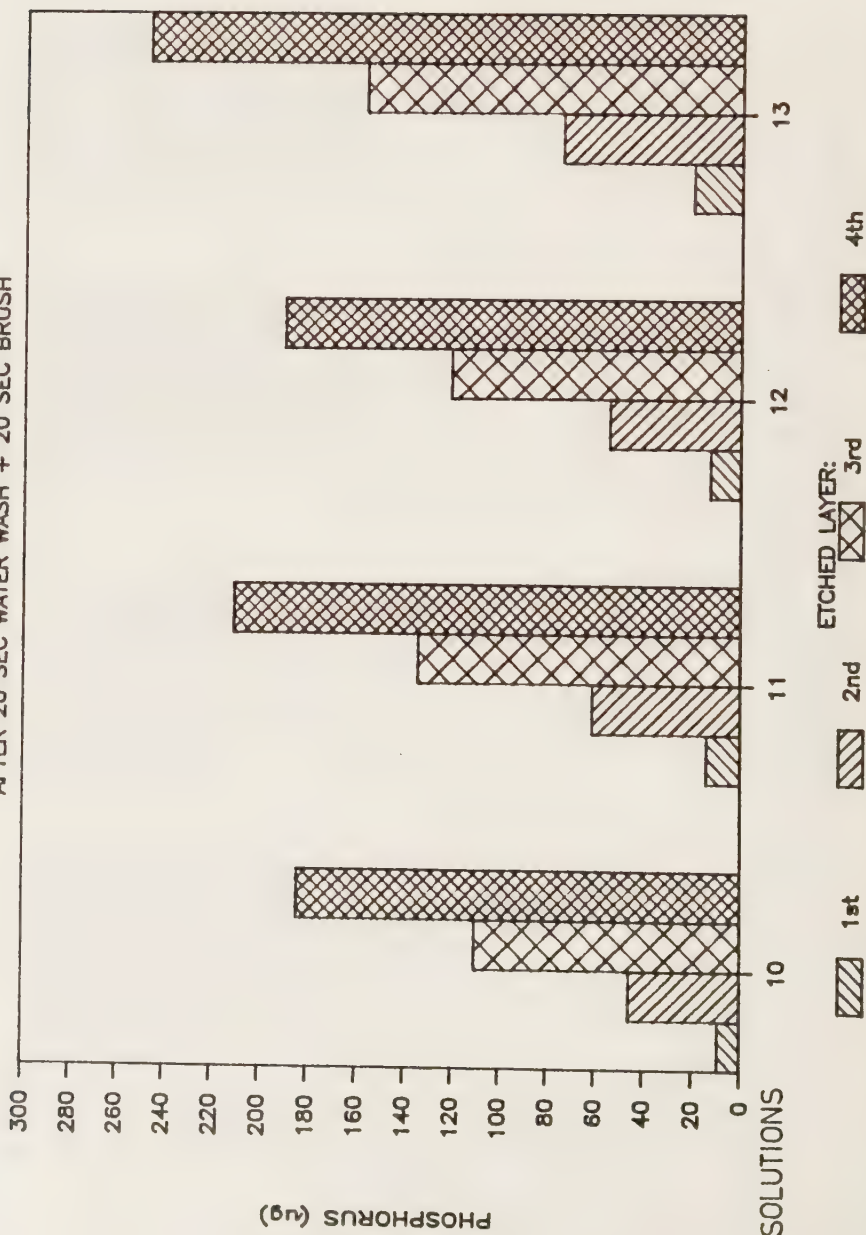


FIG. 12
ENAMEL DISSOLUTION RATE: CUMULATIVE
AFTER 20 SEC WATER WASH + 20 SEC BRUSH

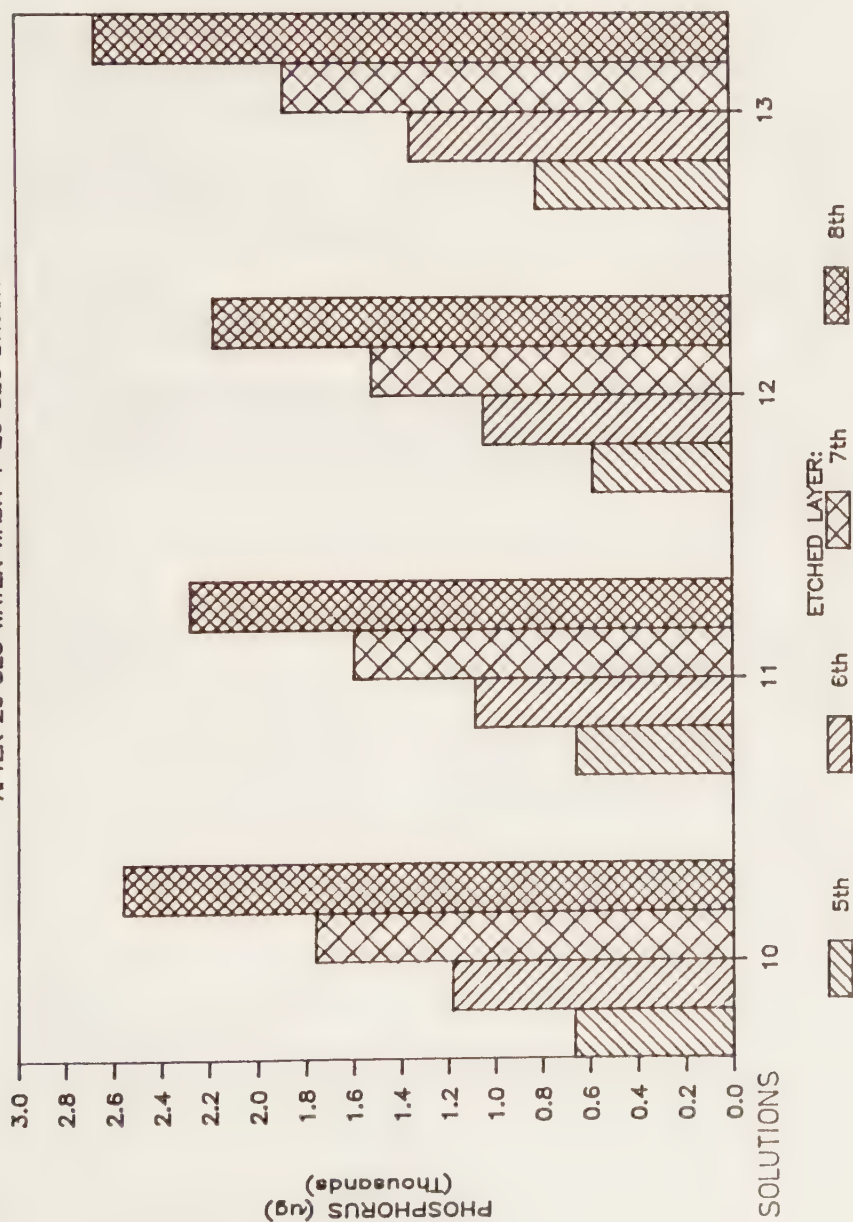


FIG. 13

FLUORIDE CONCENTRATION (Cumulative)

AFTER 10 sec WATER WASH

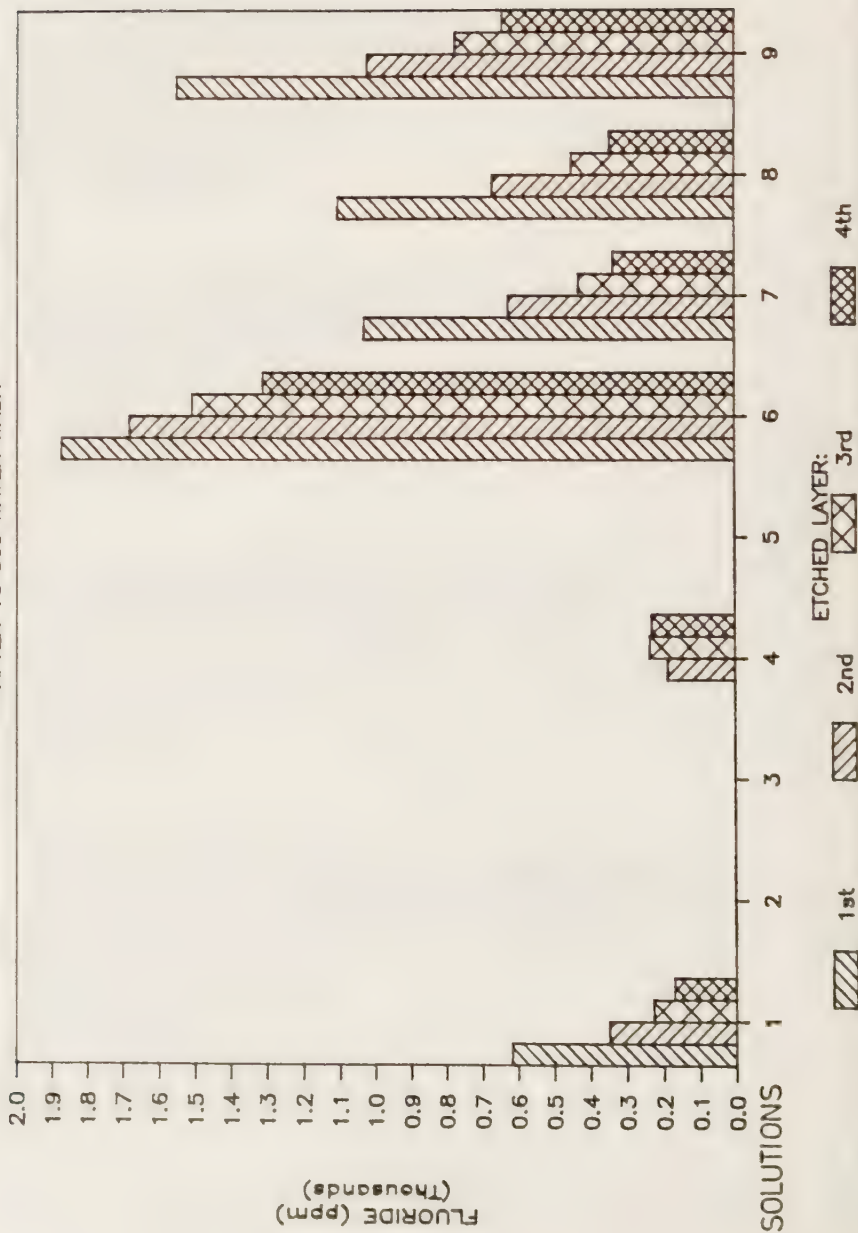
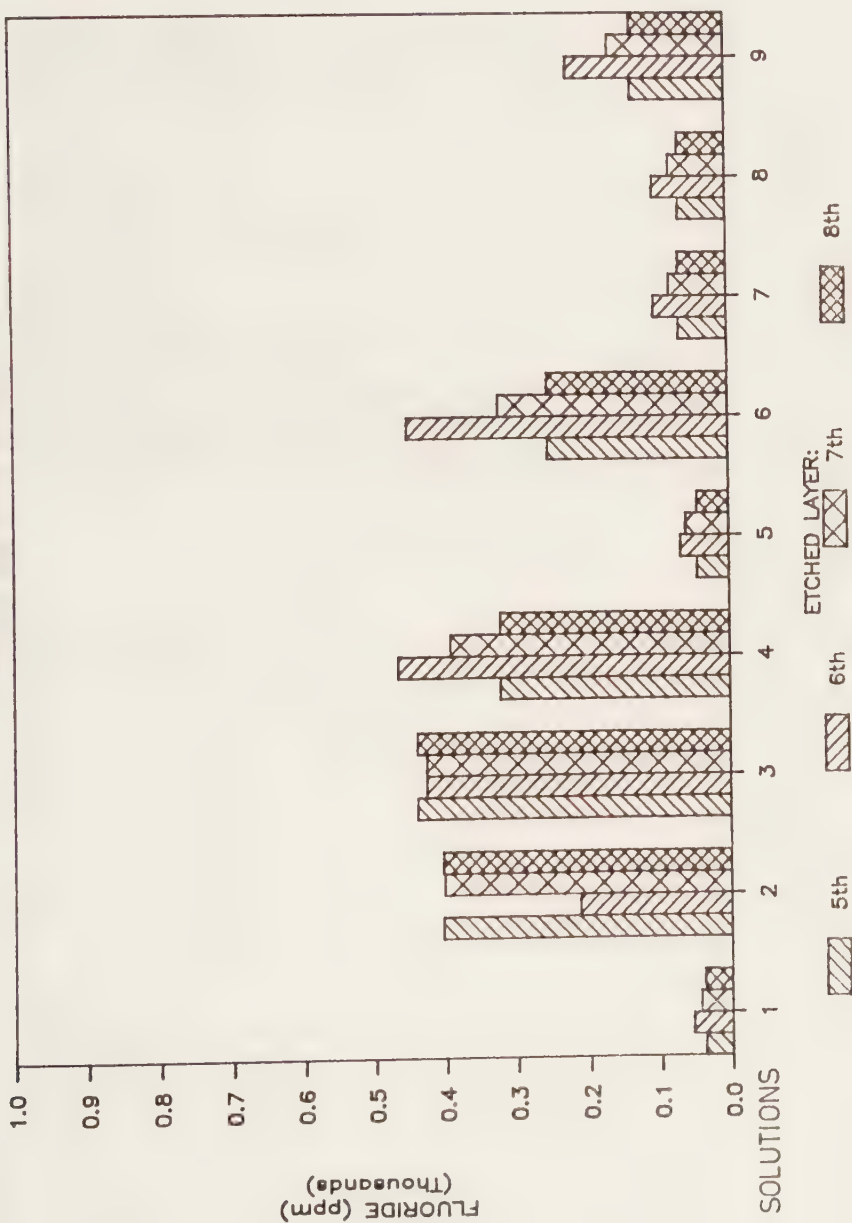


FIG. 14

FLUORIDE CONCENTRATION (Cumulative)

AFTER 10 sec WATER WASH



FLUORIDE CONCENTRATION (Cumulative)

AFTER 60 sec WATER WASH

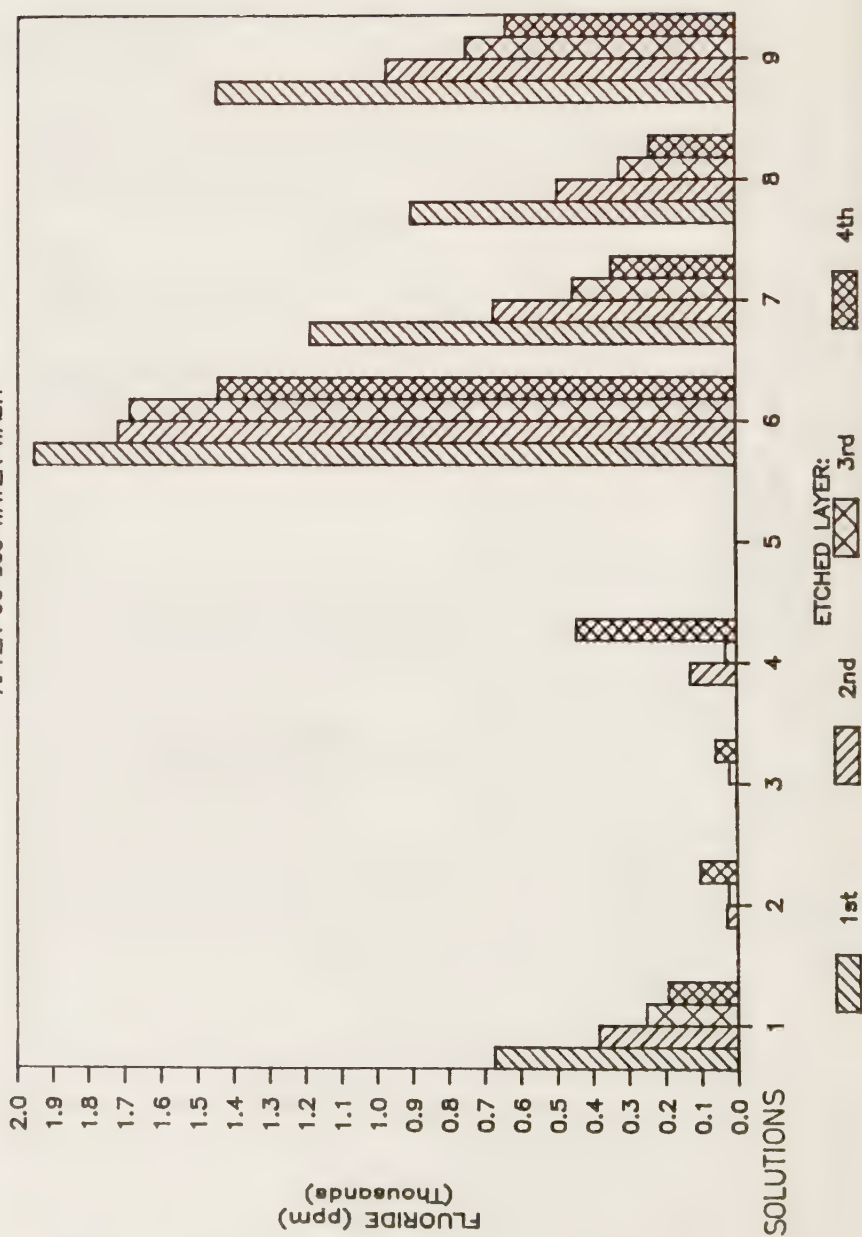


FIG. 15

FIG. 16
 FLUORIDE CONCENTRATION (Cumulative)
 AFTER 60 sec WATER WASH

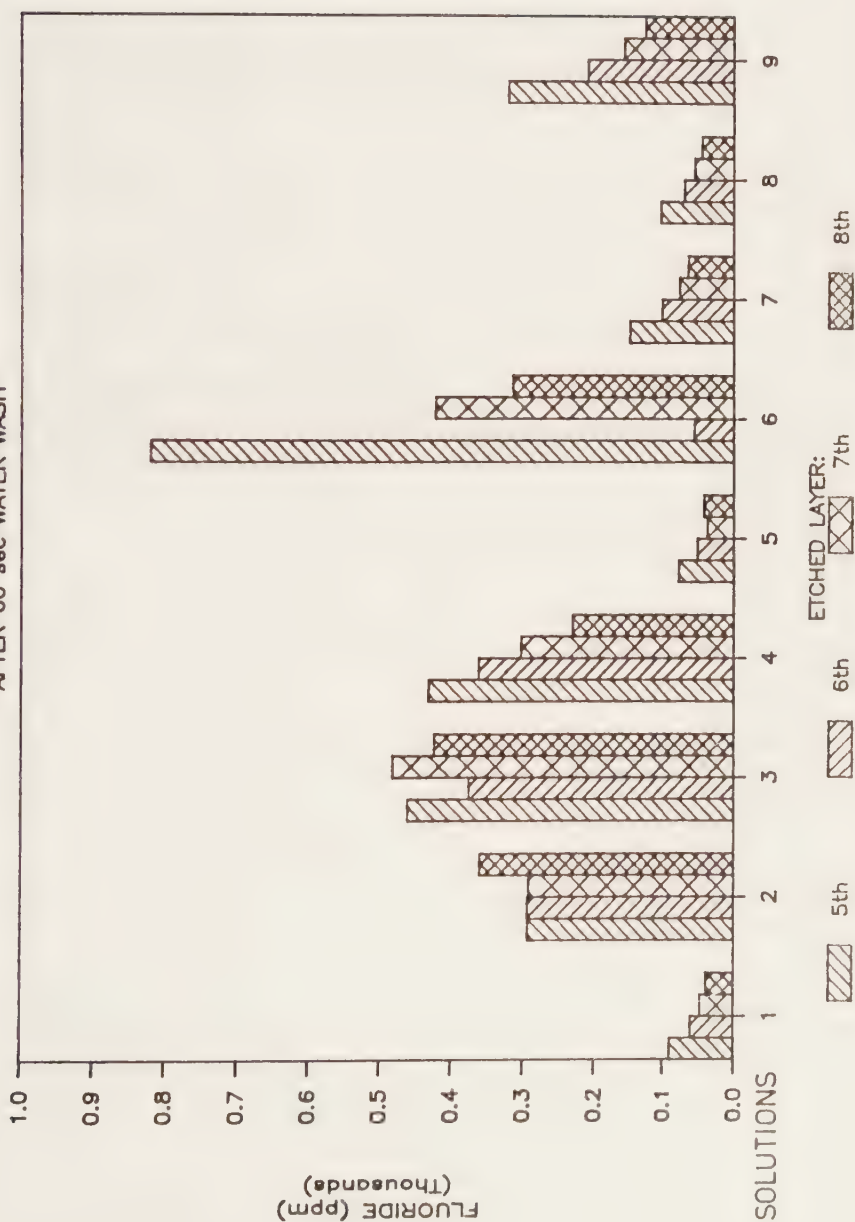


FIG. 17

FLUORIDE CONCENTRATION (Cumulative)

AFTER 5 min WATER WASH

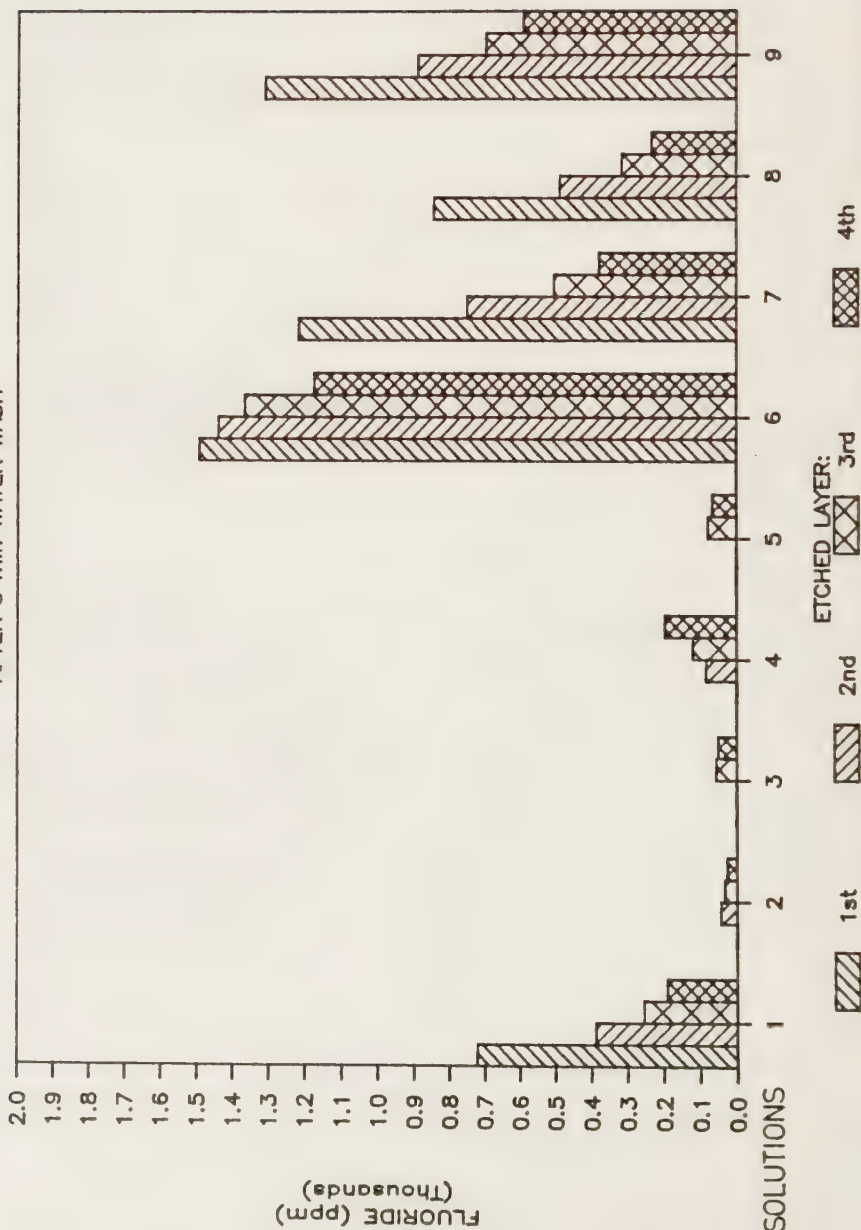
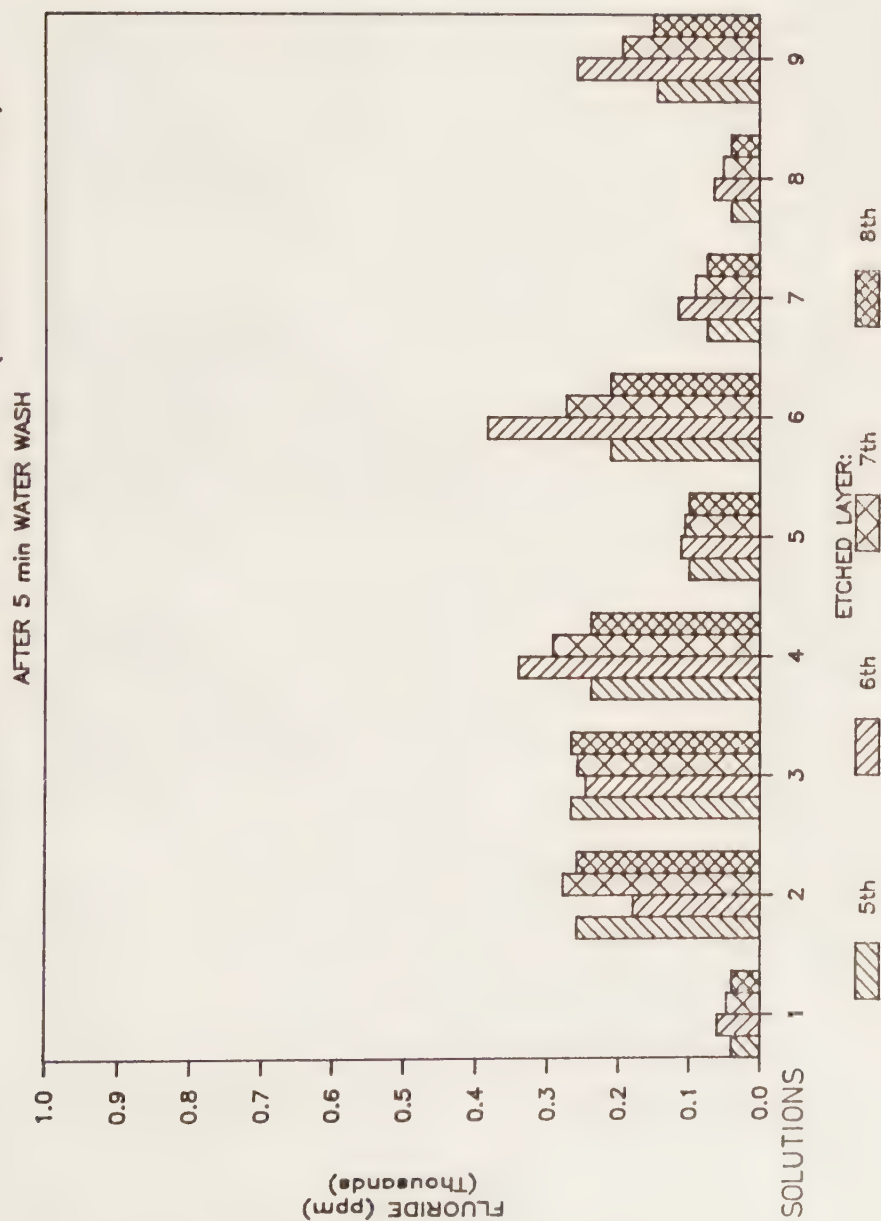


FIG. 18
FLUORIDE CONCENTRATION (Cumulative)



FLUORIDE CONCENTRATION (Cumulative)

AFTER 10 sec WATER WASH

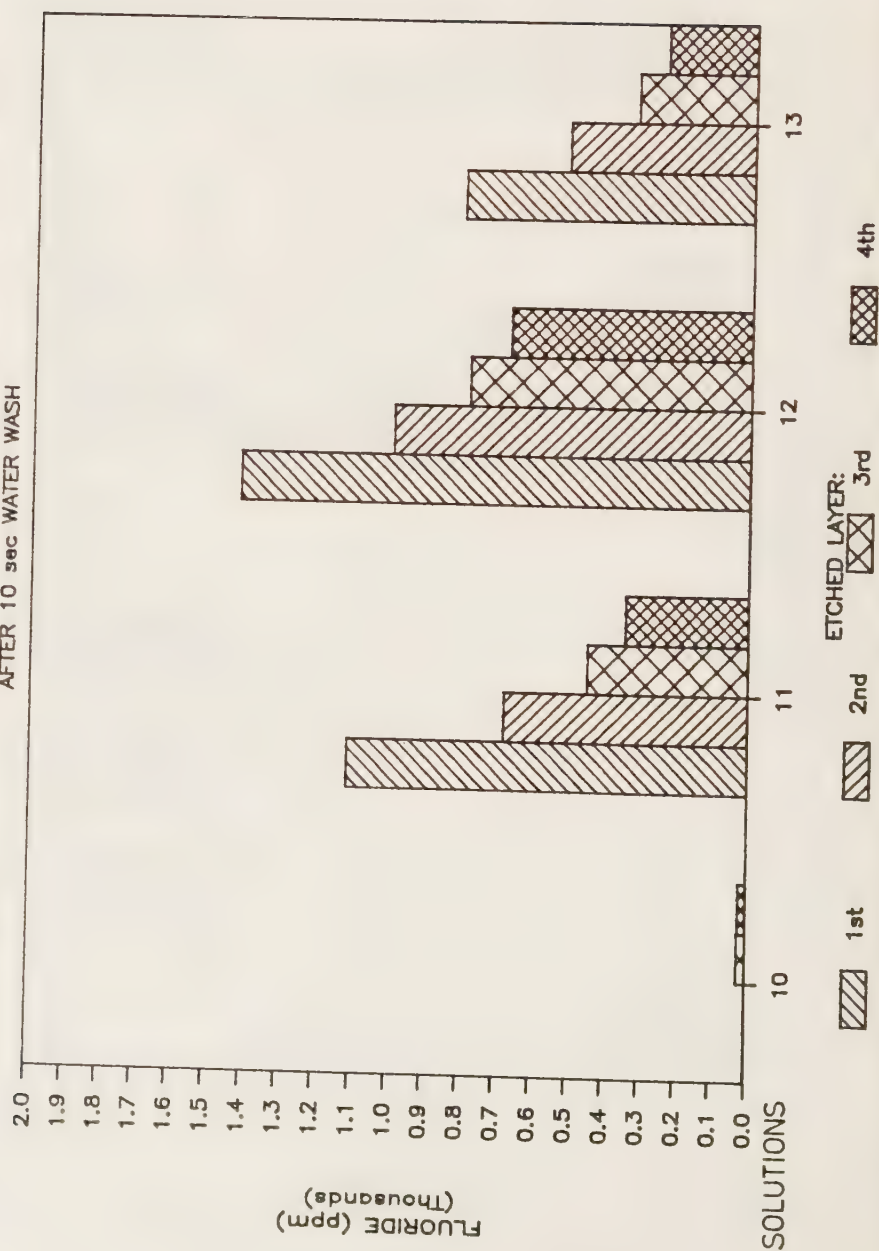


FIG. 19

FIG. 20 FLUORIDE CONCENTRATION (Cumulative)

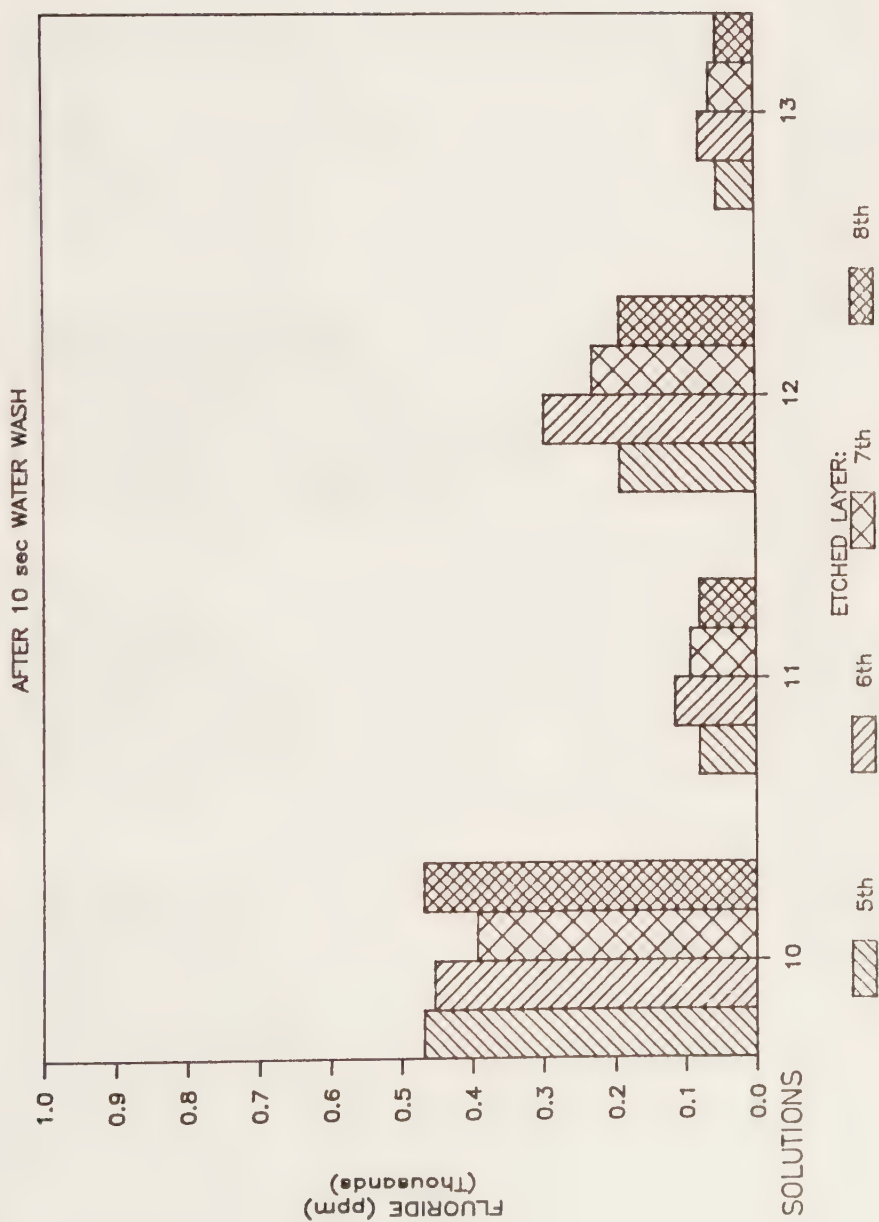
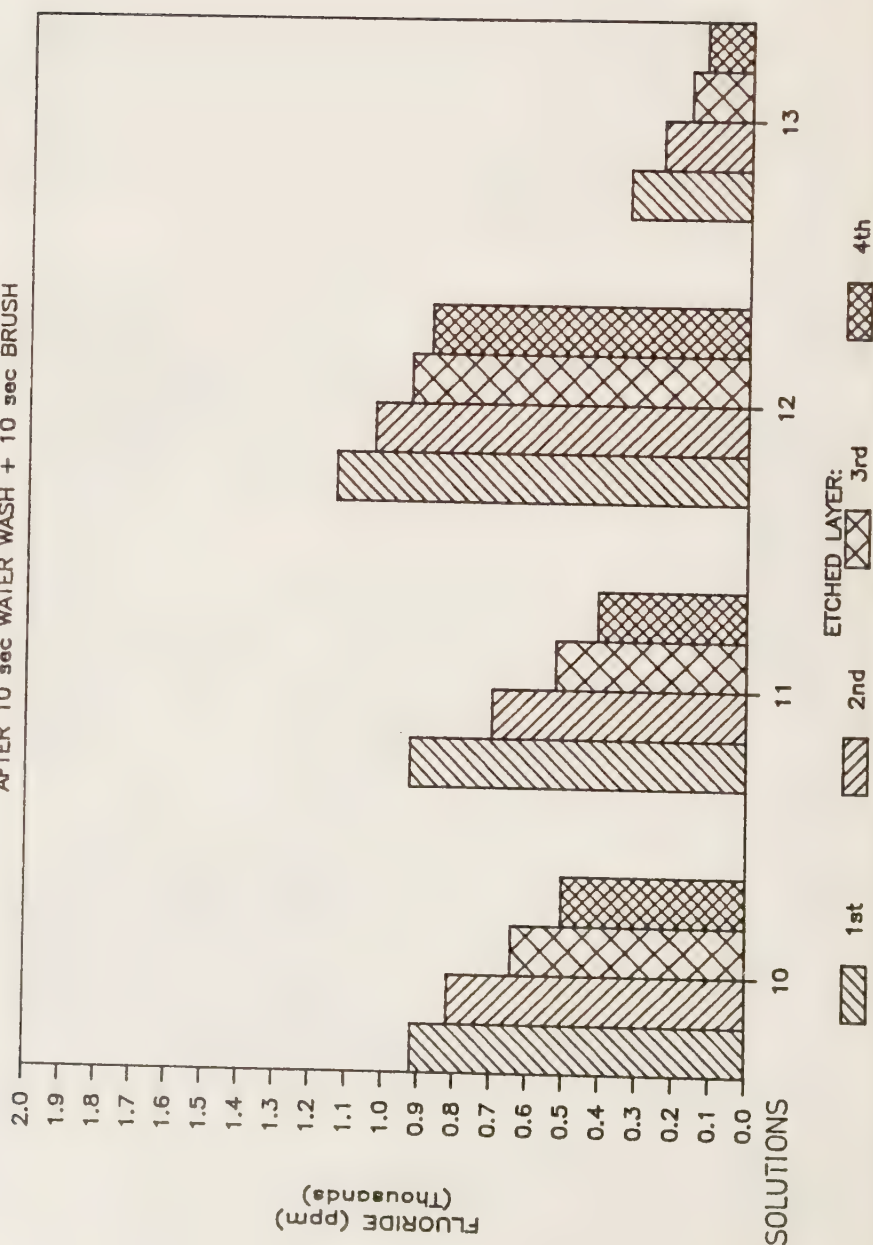


FIG. 21

FLUORIDE CONCENTRATION (Cumulative)

AFTER 10 sec WATER WASH + 10 sec BRUSH



FLUORIDE CONCENTRATION (Cumulative)

AFTER 10 sec WATER WASH + 10 sec BRUSH

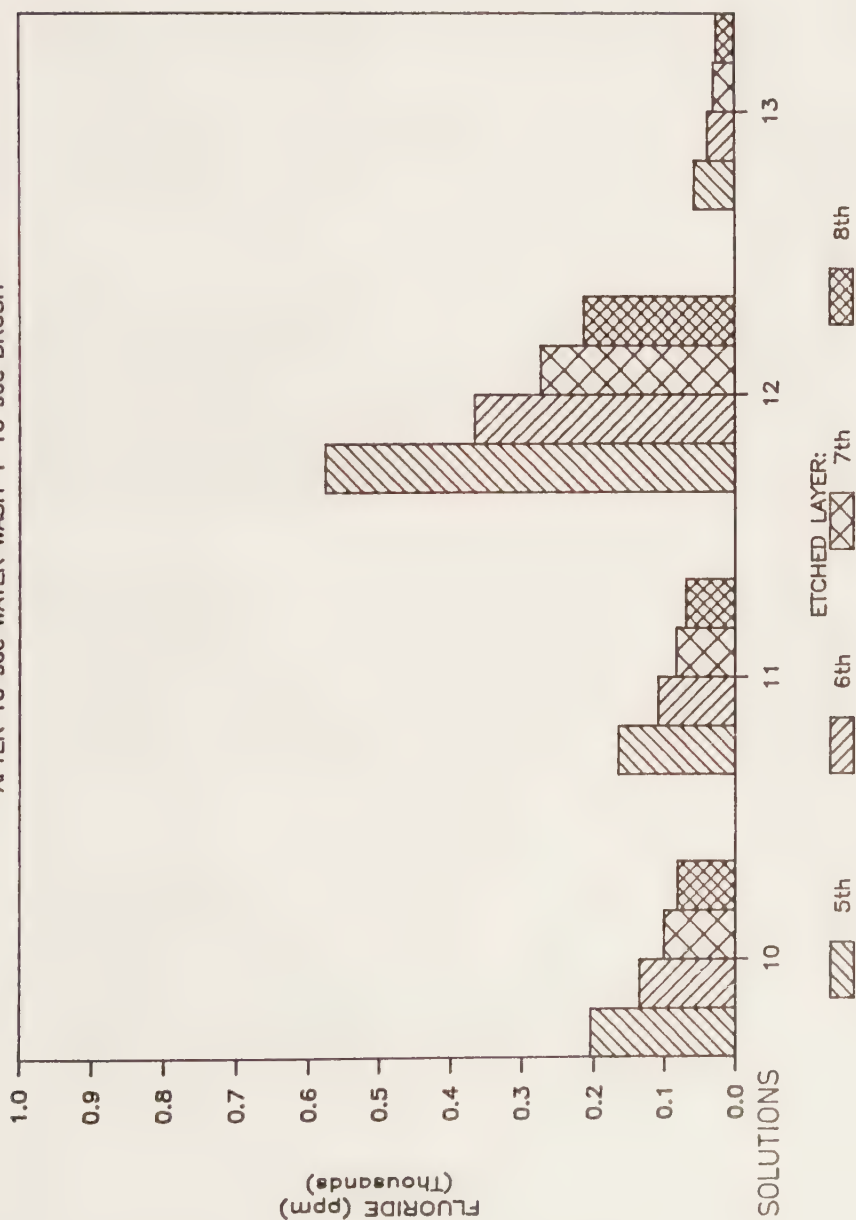


FIG. 22

FIG. 23

FLUORIDE CONCENTRATION (Cumulative)

AFTER 20 sec WATER WASH + 20 sec BRUSH

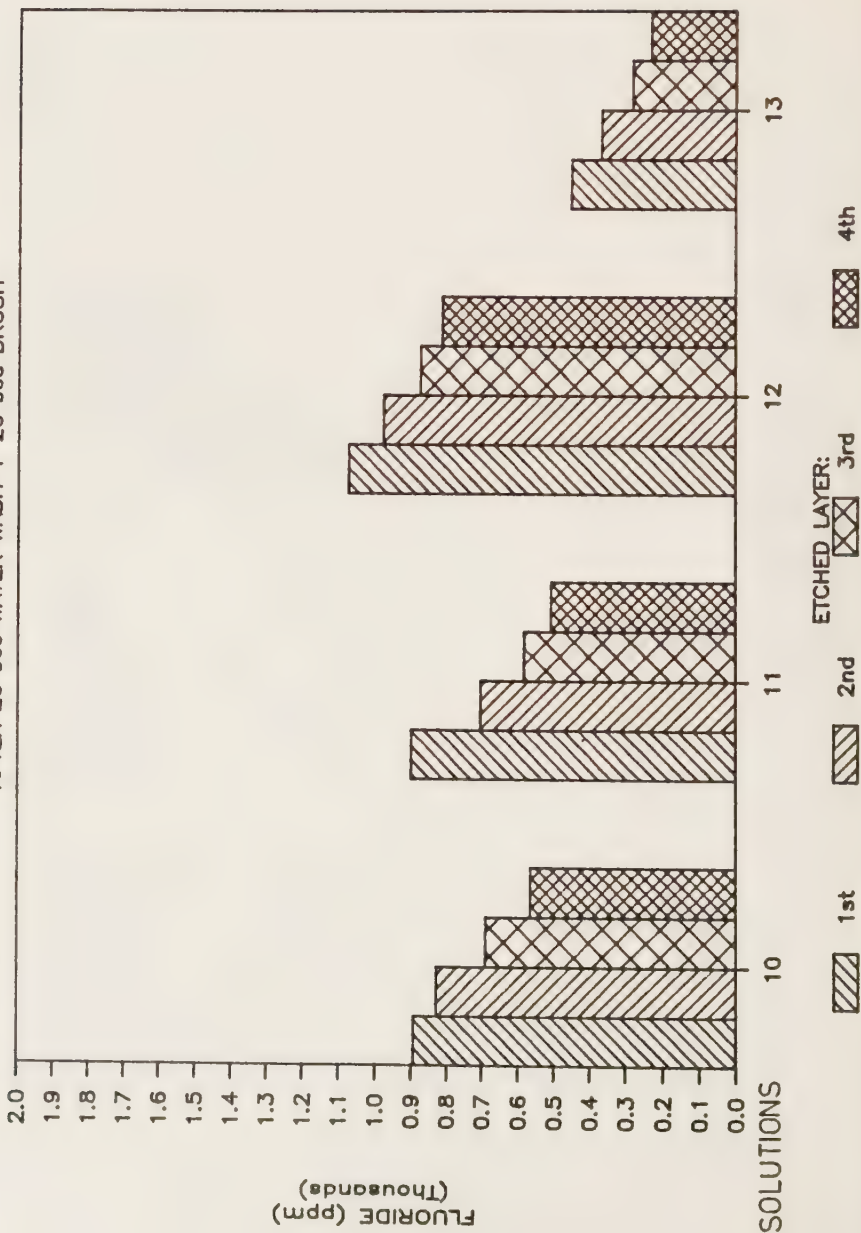


FIG. 24 FLUORIDE CONCENTRATION (Cumulative)

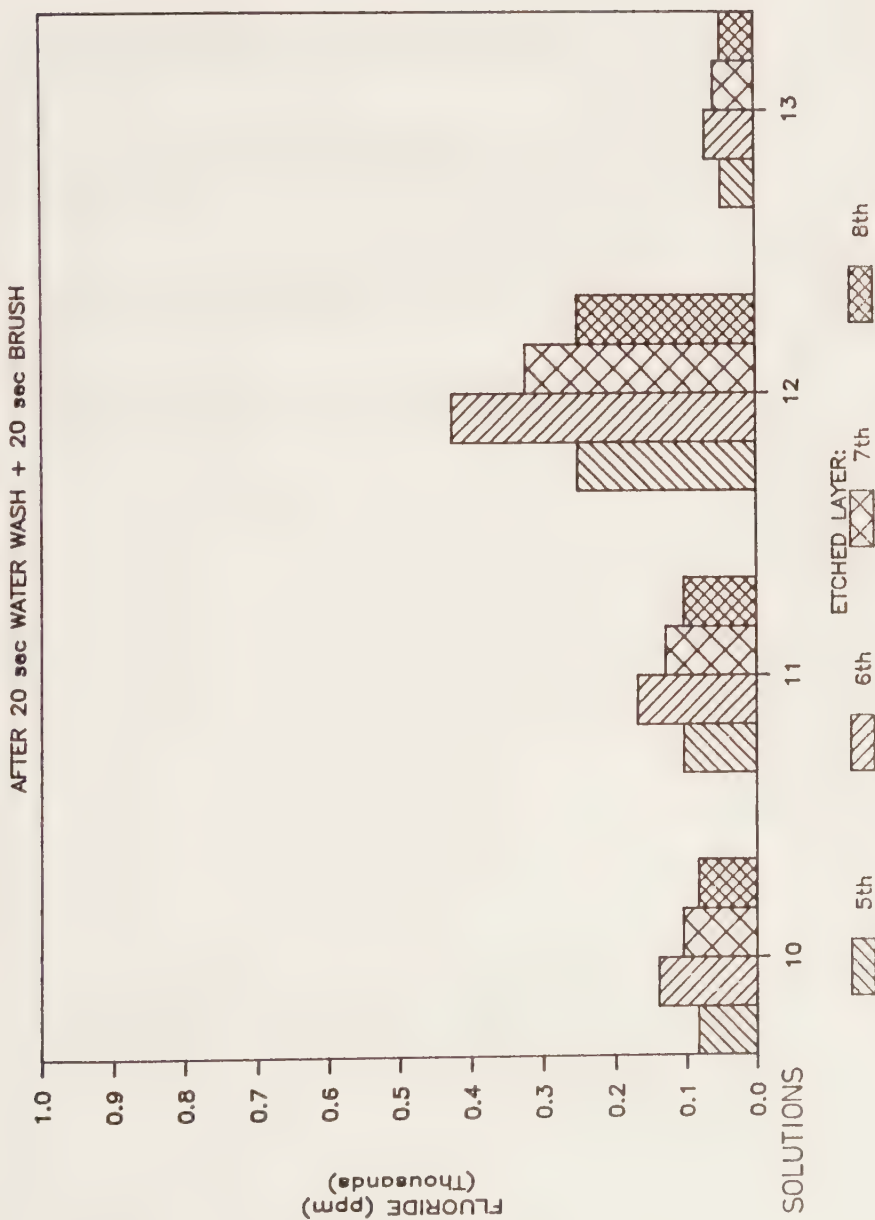


FIG. 25

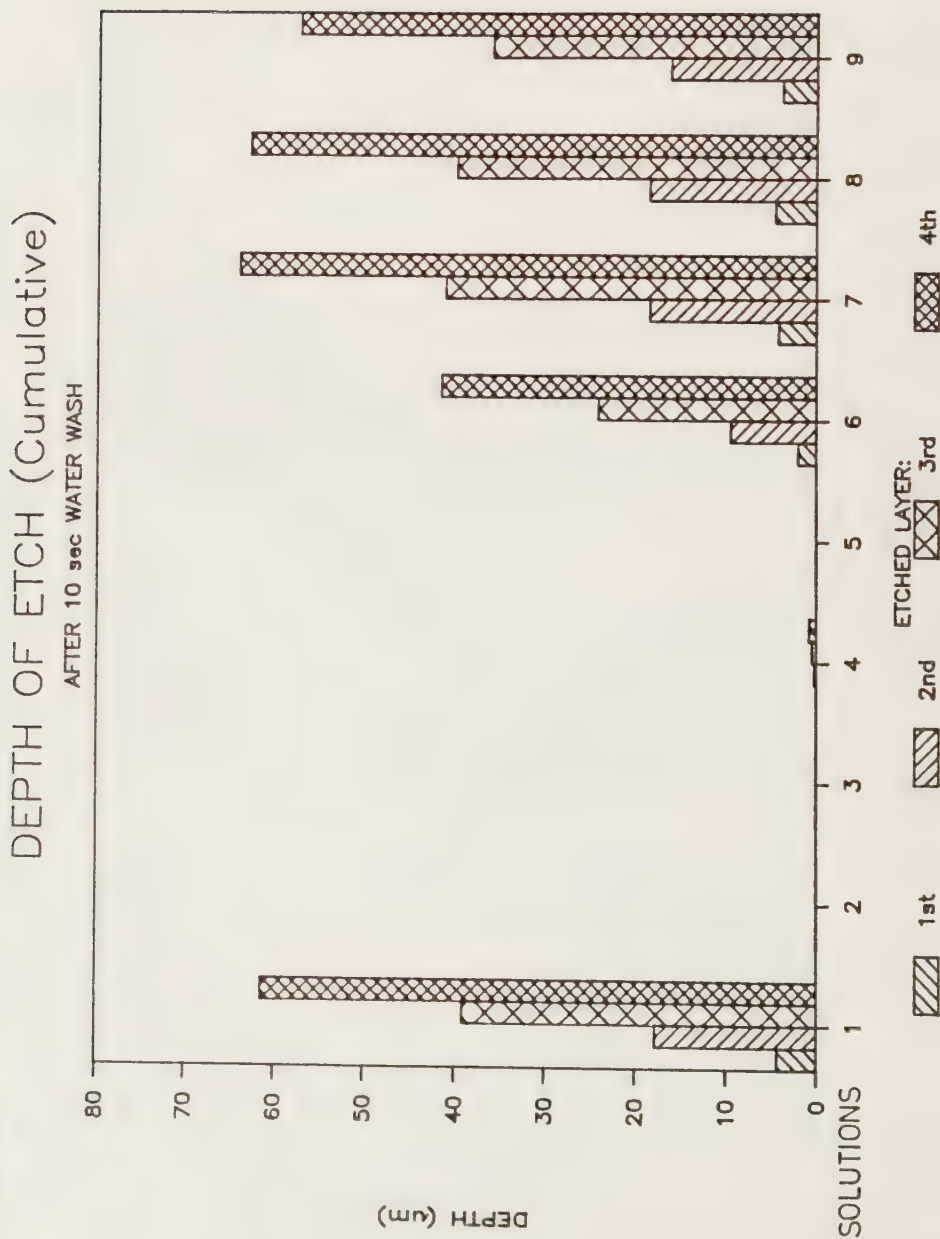


FIG. 26

DEPTH OF ETCH (Cumulative)

AFTER 10 sec WATER WASH

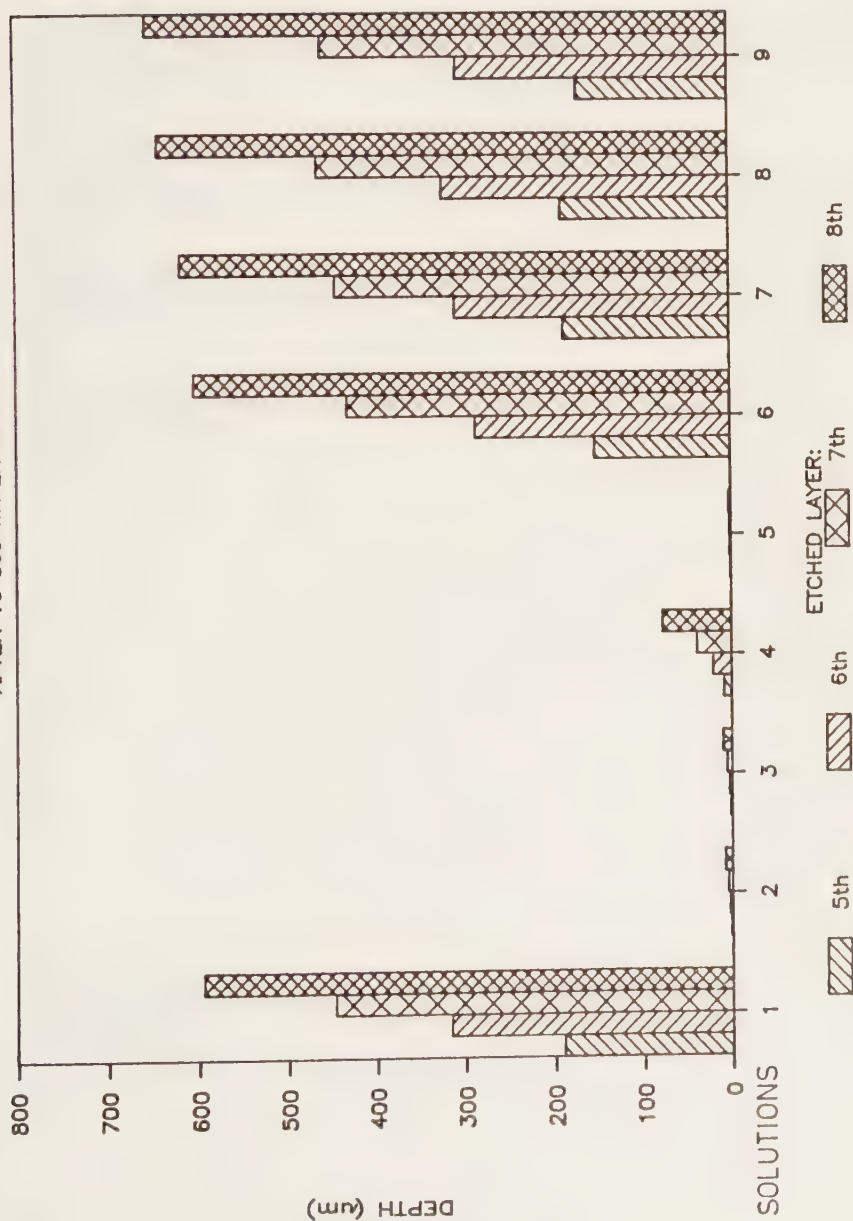


FIG. 27

DEPTH OF ETCH (Cumulative)

AFTER 60 sec WATER WASH

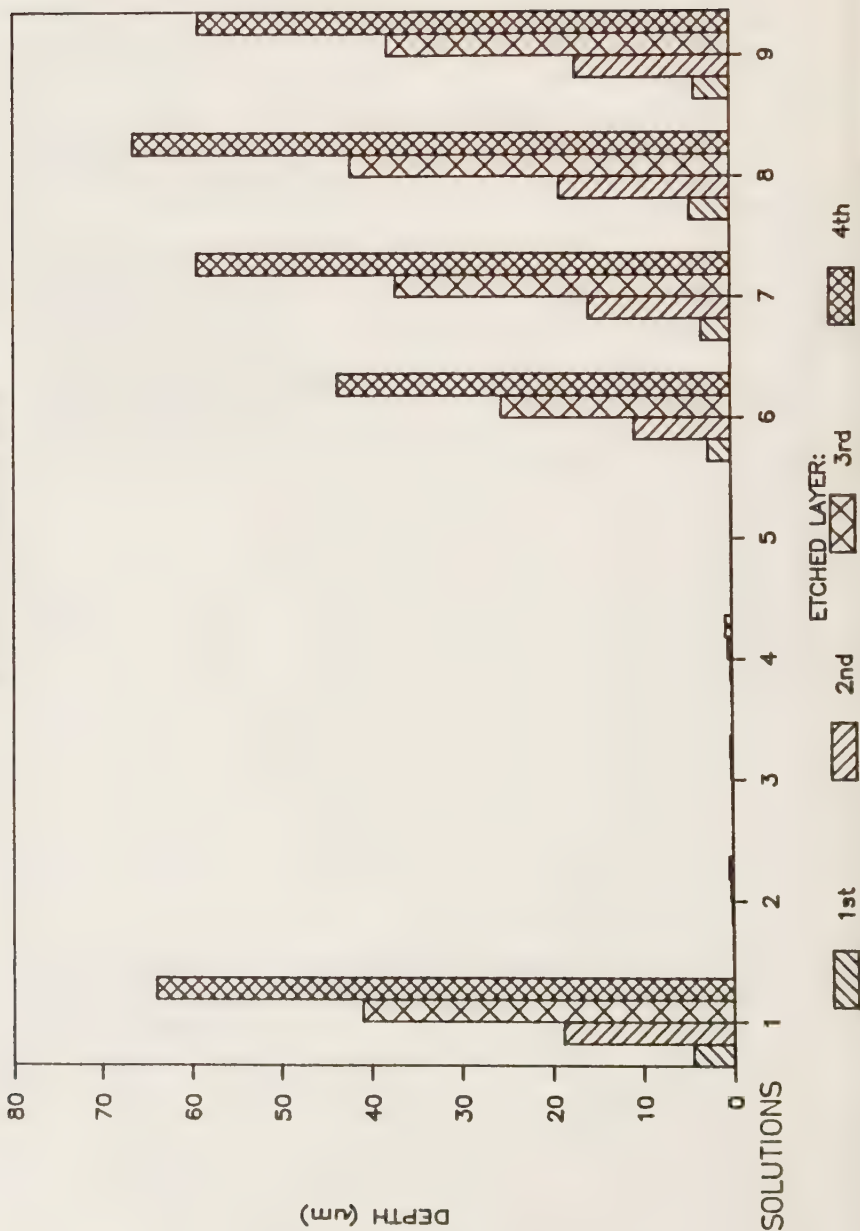
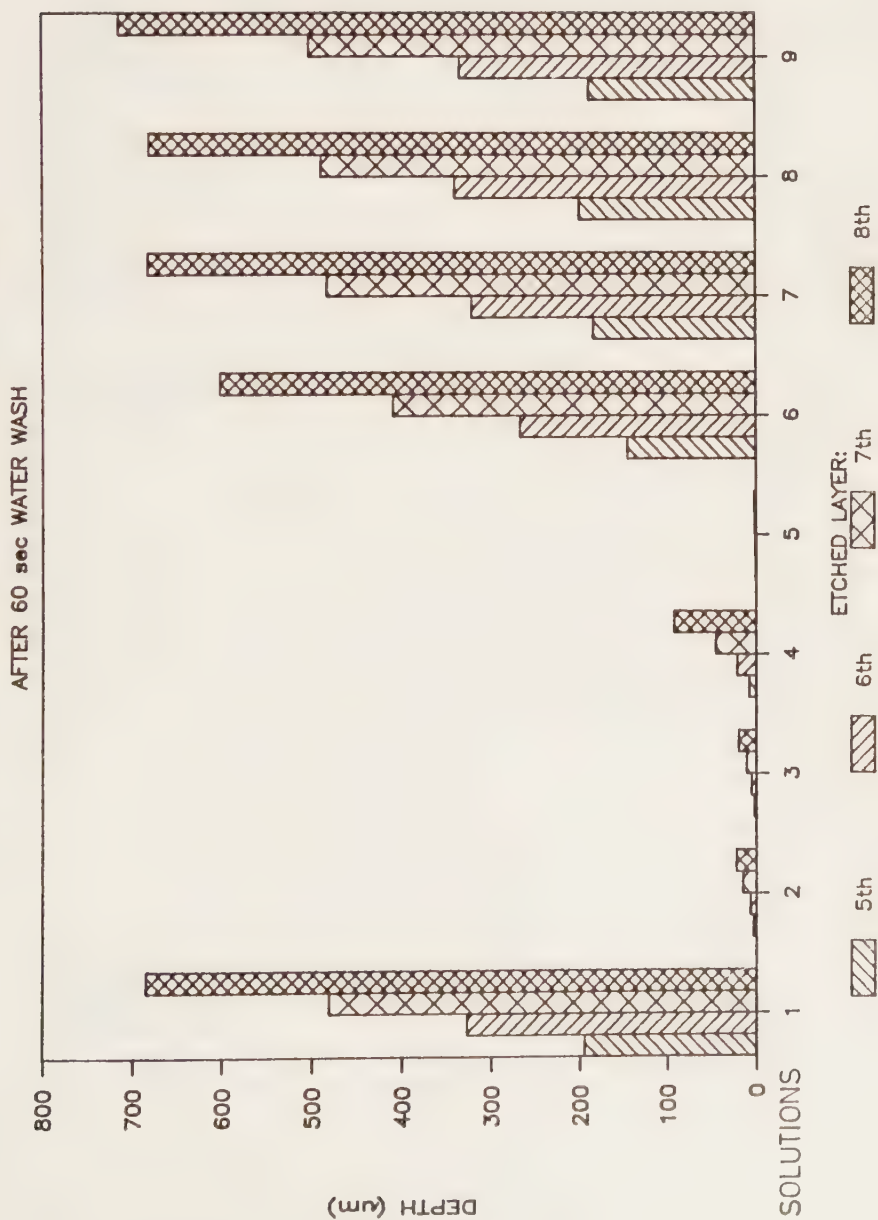


FIG. 28
DEPTH OF ETCH (Cumulative)



DEPTH OF ETCH (Cumulative)

AFTER 5 min WATER WASH

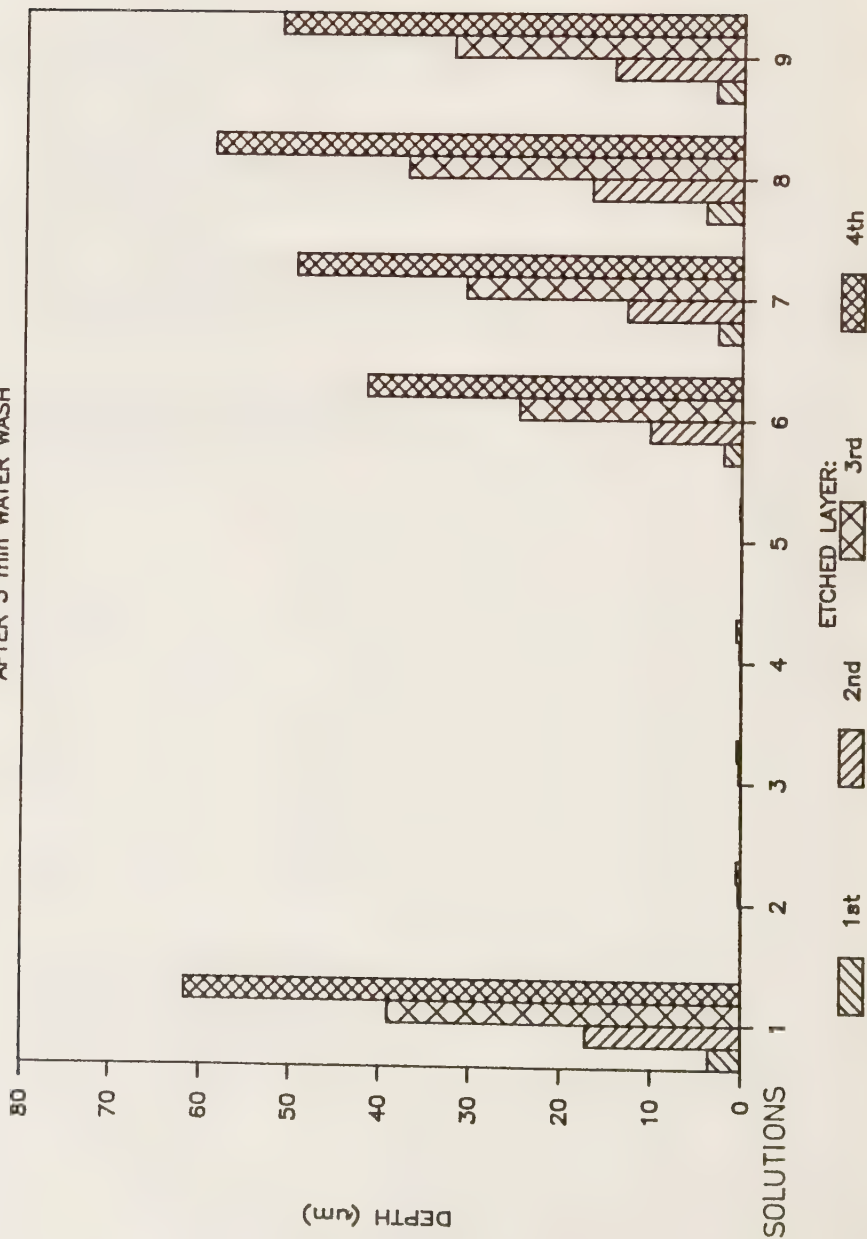
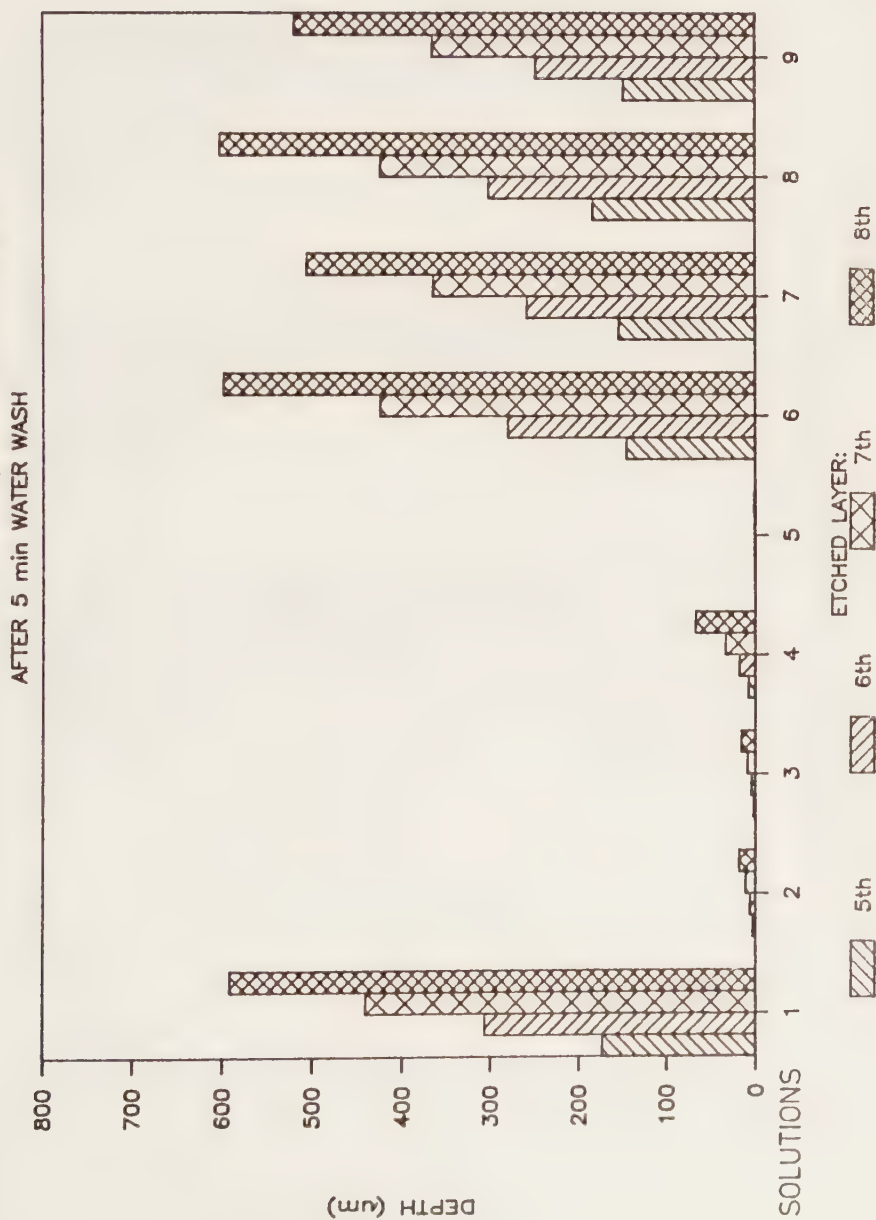


FIG. 30
DEPTH OF ETCH (Cumulative)



DEPTH OF ETCH (Cumulative)

AFTER 10 sec WATER WASH

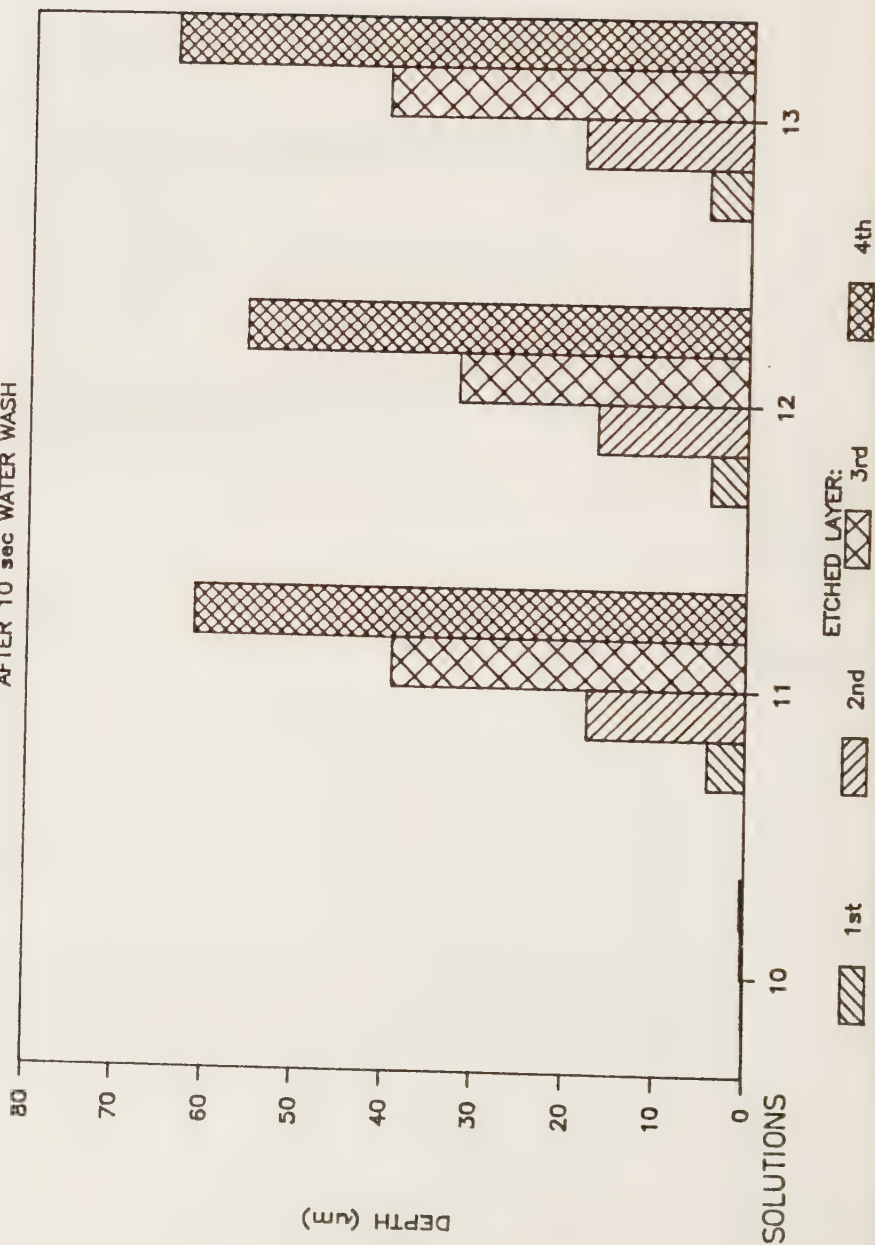
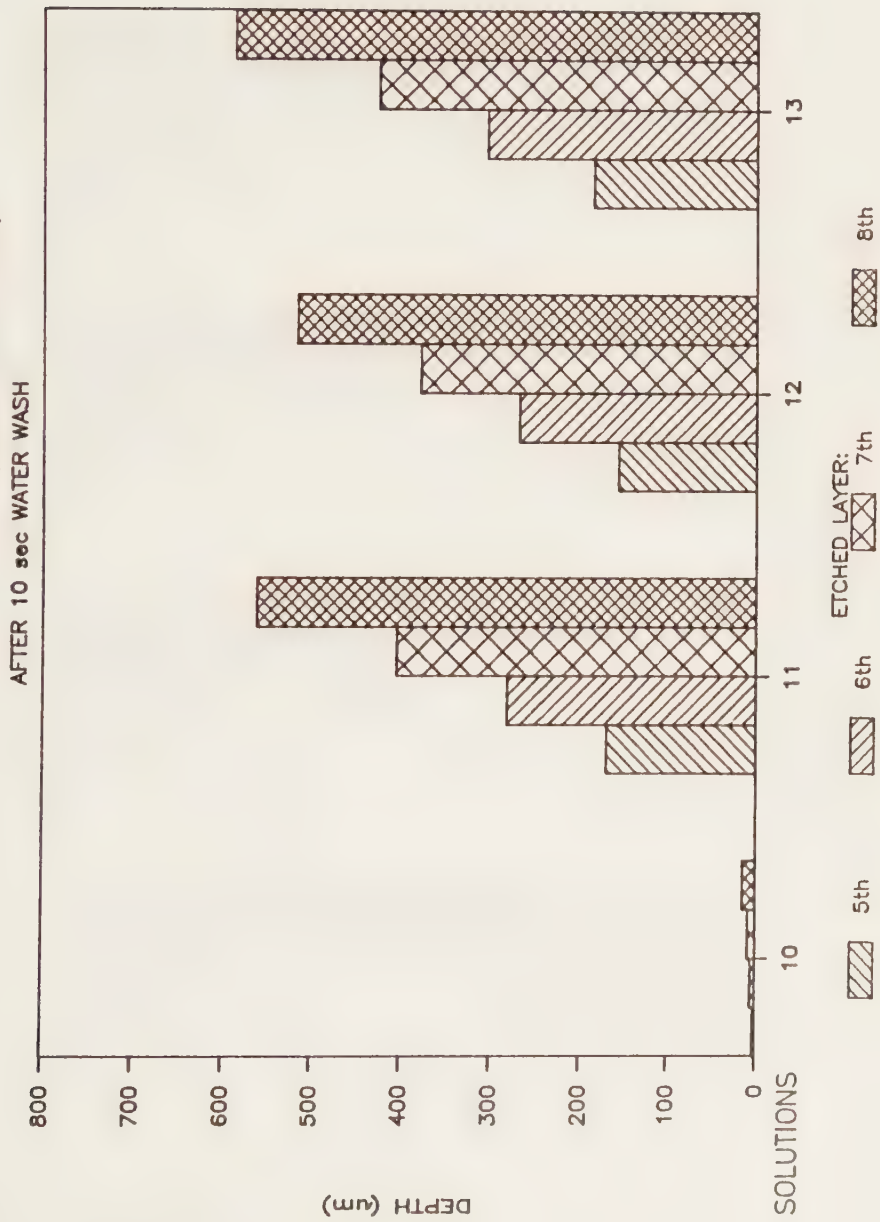


FIG. 31

FIG. 32
DEPTH OF ETCH (Cumulative)



DEPTH OF ETCH (Cumulative)

AFTER 10 sec WATER WASH + 10 sec BRUSH

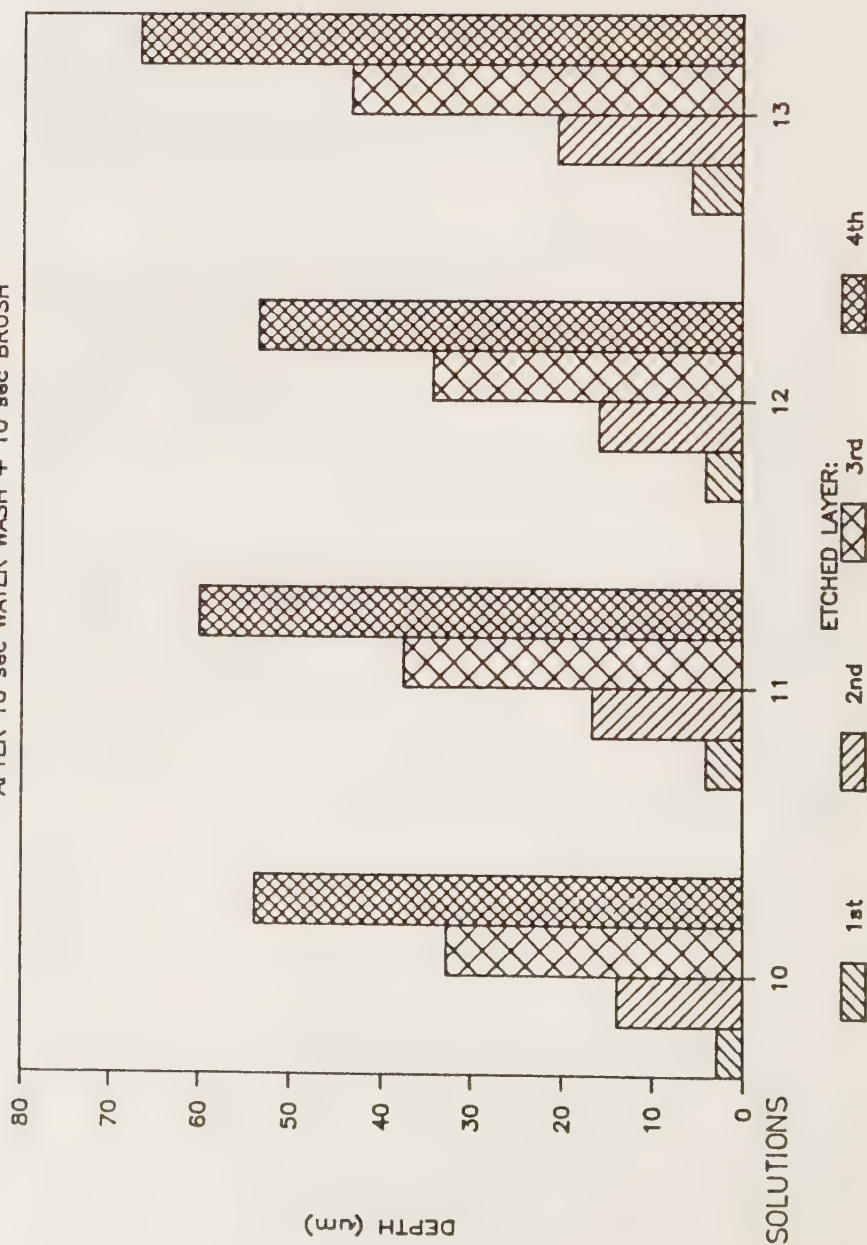
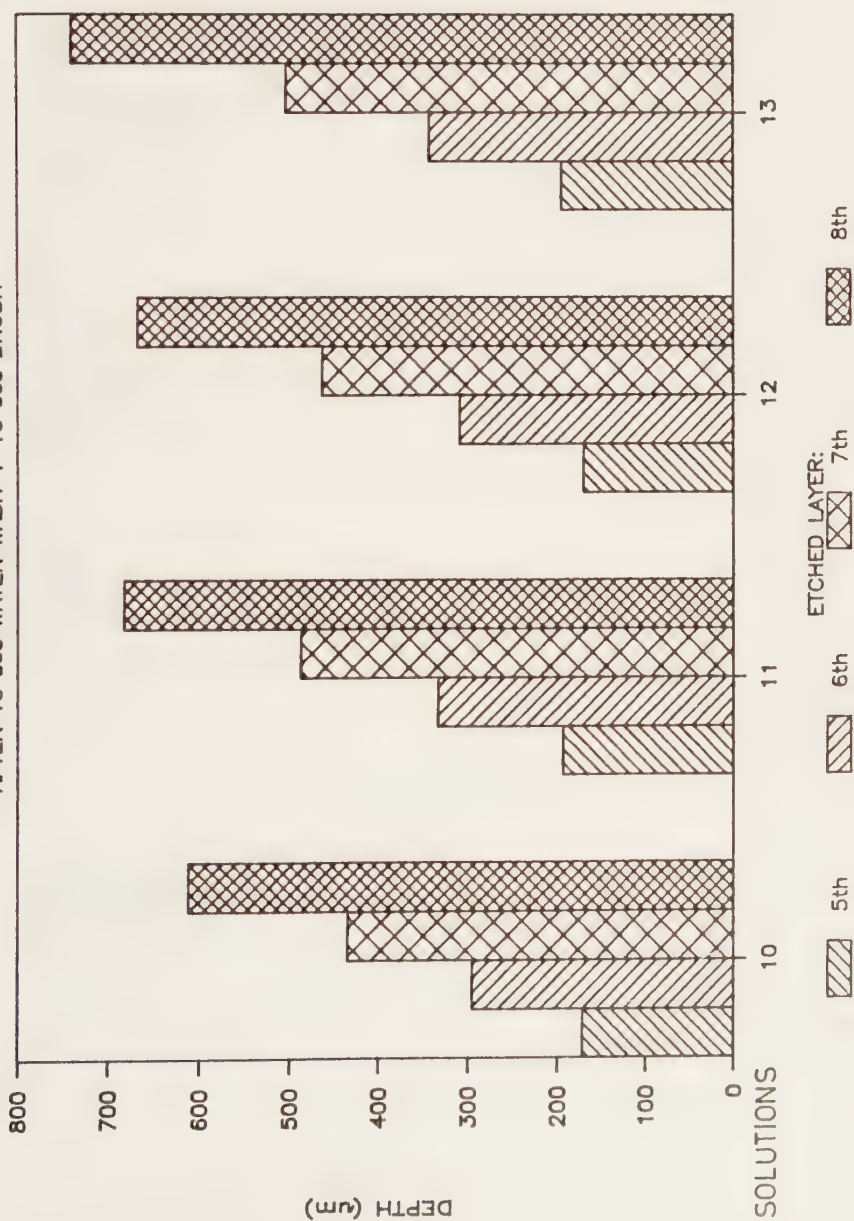


FIG. 34
DEPTH OF ETCH (Cumulative)
AFTER 10 sec WATER WASH + 10 sec BRUSH



DEPTH OF ETCH (Cumulative)

AFTER 20 sec WATER WASH + 20 sec BRUSH

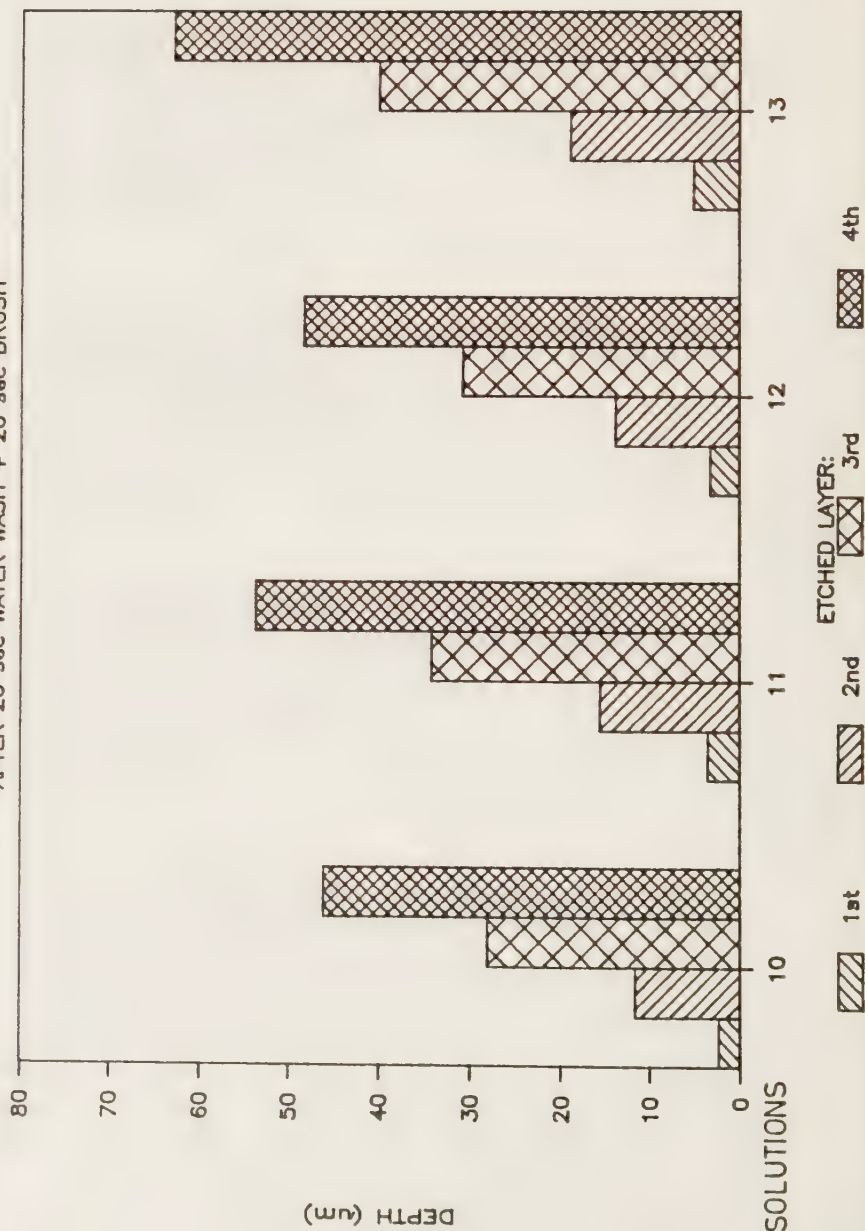


FIG. 35

FIG. 36
DEPTH OF ETCH (Cumulative)
AFTER 20 sec WATER WASH + 20 sec BRUSH



16. CURRICULUM VITAE

1959	Date of Birth: May 9th, 1959 in Uzwil.
1966 - 1972	Elementary School in Aadorf.
1972 - 1979	High-School in Frauenfeld.
1979	Matriculation Type B in Frauenfeld.
1979 - 1982	Pre-Clinical Studies at the University of Zürich.
1982 - 1986	Clinical Studies at the University of Zürich.
1986	Federal Diploma as Dental Surgeon at the University of Zürich; 17 th October, 1986.
1986	General Dental Practice in Bellinzona, Switzerland.

